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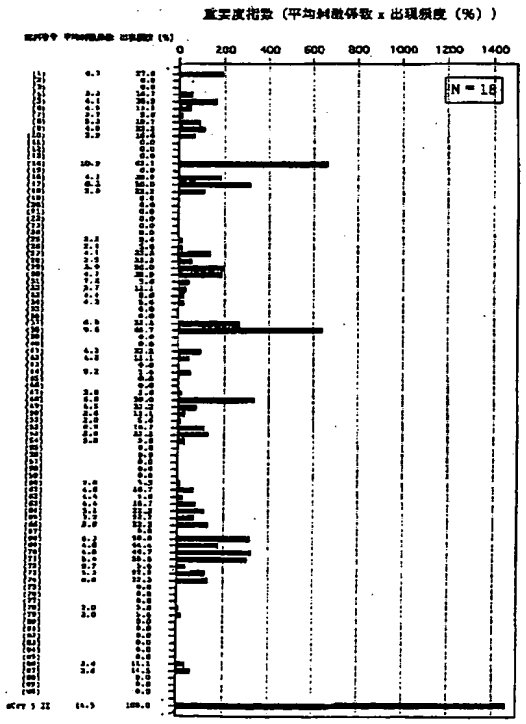
(54)【発明の名称】　スギ花粉アレルゲンＣｒｙ　ｊ　ＩＩエピトープ

(57)【要約】

【目的】　スギ花粉症の診断、予防および治療に有用なスギ花粉アレルゲンCryj IIの少なくとも一つのエピトープ、特にT 細胞エピトープを含むタンパク質またはペプチドを提供する。

【構成】　スギ花粉アレルゲンCry j IIをコードするcDNAをクローニングし、Cry j IIの全アミノ酸配列を明らかにした。更に、該アミノ酸配列全長にわたってオーバーラップペプチドを合成し、スギ花粉症患者由来のCry j II T細胞ラインを用いて、T 細胞エピトープを含むオーバーラップペプチドを同定した。



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【特許請求の範囲】

【請求項１】　配列表１記載のアミノ酸配列の全部または一部を含み、少なくとも１つのエピトープを保持するタンパク質またはペプチド。

【請求項２】　請求項１記載のタンパク質またはペプチドをコードするＤＮＡ。

【請求項３】　配列表３記載の塩基配列の全部または一部を含むことを特徴とする、請求項２記載のＤＮＡ。

【請求項４】　配列表２記載のアミノ酸配列の全部または一部を含み、少なくとも１つのエピトープを保持するタンパク質またはペプチド。

【請求項５】　請求項４記載のタンパク質またはペプチドをコードするＤＮＡ。

【請求項６】　配列表４記載の塩基配列の全部または一部を含むことを特徴とする、請求項５記載のＤＮＡ。

【請求項７】　Ｔ細胞エピトープであることを特徴とする、請求項１または４記載のタンパク質またはペプチド。

【請求項８】　下記のそれぞれのアミノ酸配列の全部または一部を含むことを特徴とする、請求項７記載のタンパク質またはペプチド。

1. Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr Gly Ala Val Gly Asp

4. Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala

5. Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala Ala Cys Lys Asn Pro

6. Thr Ala Trp Gln Ala Ala Cys Lys Asn Pro Ser Ala Met Leu Leu

7. Ala Cys Lys Asn Pro Ser Ala Met Leu Leu Val Pro Gly Ser Lys

8. Ser Ala Met Leu Leu Val Pro Gly Ser Lys Lys Phe Val Val Asn

9. Val Pro Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn

10. Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys Gln Pro

14. Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys

16. Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys

17. Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr

18. Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly Lys Gly

25. Glu Ile Cys Asn Asp Arg Asp Arg Pro Thr Ala Ile Lys Phe Asp

26. Arg Asp Arg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu

27. Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu

28. Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser

29. Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His Leu

30. Lys Leu Met Asn Ser Pro Glu Phe His Leu Val Phe Gly Asn Cys

31. Pro Glu Phe His Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile

32. Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile

33. Glu Gly Val Lys Ile Ile Gly Ile Ser Ile Thr Ala Pro Arg Asp

34. Ile Gly Ile Ser Ile Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp

37. Gly Ile Asp Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn

38. Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly

41. Asp Asp Cys Val Ala Ile Gly Thr Gly Ser Ser Asn Ile Val Ile

42. Ile Gly Thr Gly Ser Ser Asn Ile Val Ile Glu Asp Leu Ile Cys

44. Glu Asp Leu Ile Cys Gly Pro Gly His Gly Ile Ser Ile Gly Ser

47. Leu Gly Arg Glu Asn Ser Arg Ala Glu Val Ser Tyr Val His Val

48. Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe

49. Ser Tyr Val His Val Asn Gly Ala Lys Phe Ile Asp Thr Gln Asn

50. Asn Gly Ala Lys Phe Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys

51. Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly

52. Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser Gly Met Ala Ser

53. Thr Trp Gln Gly Gly Ser Gly Met Ala Ser His Ile Ile Tyr Glu

54. Ser Gly Met Ala Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile

60. Ala Ser Ala Cys Gln Asn Gln Arg Ser Ala Val Gln Ile Gln Asp

61. Asn Gln Arg Ser Ala Val Gln Ile Gln Asp Val Thr Tyr Lys Asn

62. Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser

63. Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala

64. Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Ile Gl

n Leu Lys Cys
65. Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser As p Ser Met Pro
66. Ile Gln Leu Lys Cys Ser Asp Ser Met Pro Cys Ly s Asp Ile Lys
68. Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu Ly s Leu Thr Ser
69. Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser Gly Ly s Ile Ala Ser
70. Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Le u Asn Asp Asn
71. Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala As n Gly Tyr Phe
72. Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gl y His Val Ile
73. Ala Asn Gly Tyr Phe Ser Gly His Val Ile Pro Al a Cys Lys Asn
74. Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Se r Pro Ser Ala
78. Lys Ser His Lys His Pro Lys Thr Val Met Val Gl u Asn Met Arg
79. Pro Lys Thr Val Met Val Glu Asn Met Arg Ala Ty r Asp Lys Gly
86. Cys Ser Pro Cys Lys Ala Lys Leu Val Ile Val Hi s Arg Ile Met
87. Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gl n Glu Tyr Tyr

【請求項９】　下記のそれぞれのアミノ酸配列の全部または一部を含むことを特徴とする、請求項７記載のタンパク質またはペプチド。
14. Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Al a Ser Trp Lys
17. Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Th r Gly Phe Thr
29. Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Gl u Phe His Leu
38. Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Il e Gly Thr Gly
48. Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gl y Ala Lys Phe
68. Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu Ly s Leu Thr Ser
70. Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Le u Asn Asp Asn
71. Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala As n Gly Tyr Phe

【請求項１０】　請求項８または９に記載のタンパク質またはペプチドをコードするＤＮＡ。

【請求項１１】　請求項８または９に記載のタンパク質またはペプチドと免疫学的に交差反応性を有するタンパク質またはペプチド。

【請求項１２】　請求項１１に記載のタンパク質またはペプチドをコードするＤＮＡ。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、スギ花粉症の診断、予防もしくは治療に有用な、スギ花粉アレルゲンCry j II のエピトープ、特にT 細胞エピトープを含むタンパク質またはペプチド、または該タンパク質またはペプチドをコードするＤＮＡに関する。

【０００２】

【従来の技術】スギ花粉症は、スギ花粉が飛散する春先にほぼ全国的に観察されるアレルギー性疾患であり、くしゃみや鼻汁、目のかゆみ等を伴うアレルギー症状を呈する。その患者数は、1970年以降急激に増加しており、現在全国民の10% 弱に当たる約一千万人がスギ花粉症に苦しめられている。

【０００３】アレルギー性疾患を形成するアレルギー反応は、R. G. H. Gell とR. R. A. Coombs によりＩ型～IV型の4 種に分類されており、スギ花粉症はＩ型に属する。Ｉ型アレルギーの発症機序は以下の通りである。

【０００４】アレルギー反応を引き起こす分子をアレルゲン（本明細書では抗原ともいう）というが、花粉の場合このアレルゲンがタンパク質抗原である。これらの外来タンパク質抗原が体内に侵入すると、抗原提示細胞（マクロファージ）に取込まれ、タンパク分解酵素によって分解されてペプチド断片になり、主要組織適合抗原複合体（Major Histocompatibility Complex: MHC ）クラスII分子（ヒトではHLA クラスII分子）と結合した状態で、細胞膜上に提示される。HLA クラスII分子は多型性を示すが、CD4 ＋ Ｔ細胞のレセプターは、HLA クラスII分子と結合した抗原ペプチドを、そのHLA クラスII分子の多型性を示す部分と共に認識し、抗原特異的に活性化される。活性化されたCD4 ＋ Ｔ細胞は、Th0 細胞、Th1 / Th2 細胞に分化し、種々のサイトカインを産生する。その際、それぞれの細胞のサイトカイン産生パターンは異なっており、Th1 はIL-2、IFN γを、Th2 はIL-4、IL-5、IL-10 等を、Th0 は両者のサイトカインを産生する。

【０００５】一方、B 細胞は細胞表面にIgM あるいはIgD を表現しており、抗原を細胞内に取込むことによって活性化される。その際、Th2 から産生されるサイトカインの作用によって、活性化されたB 細胞は抗体産生細胞にまで分化増殖し、抗原特異的な免疫グロブリンE （IgE ）を産生する。このようにして産生されたIgE は、気道あるいは鼻粘膜組織中のマスト（肥満）細胞や血液中の好塩基球にIgE レセプターを介して強固に結合し、感作が成立した状態になる。

【０００６】再び、アレルゲンが体内に侵入すると、１分子のアレルゲンは、直ちにマスト細胞や好塩基球上の

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2 分子以上のIgE と結合し、架橋構造を形成する。その結果、IgE 分子と結合しているレセプター同士が会合し、これが引き金となって、細胞膜内の幾種類もの酵素が活性化され、ヒスタミンやプロスタグランジン、ロイコトリエンといった種々の化学伝達物質が細胞から放出される。これらの化学伝達物質が鼻粘膜や気道などの局所に作用して、色々なアレルギー症状を引き起こす。

【０００７】なお、T 細胞によって認識されるエピトープをT 細胞エピトープ、B 細胞によって認識されるエピトープをB 細胞エピトープという。

【０００８】アレルゲンのエピトープは、Ｉ型アレルギーの発症及び増悪に直接関与していると考えられるので、アレルゲンのエピトープを同定することは、Ｉ型アレルギーの診断、予防及び治療に有用である。

【０００９】スギ花粉の主要アレルゲンは、安枝らによって単離精製され、Sugi Basic Protein (SBP) と命名された (Yasueda, H., et al., J. Allergy Clin. Immunol.71, 77-86, 1983) 。このSBP は、分子量が45～50kDa で、WHO の命名法に従い現在Cry j I と呼ばれている。更にその後、Cry j I の分離精製の過程で、Cryj I とは抗原性の異なる、分子量が37kDa のCry j IIが分離された (Taniai, M.et al. FEBS Letters 239, 329-332,1988、Sakaguchi, M. et al. Allergy 45,309-312,1990) 。

【００１０】これらの結果、Cry j I とCry j IIとは全く異なるタンパクであることが明らかとなったが、スギ花粉症患者では、Cry j I とCry j IIの両者が反応していることが報告された。すなわち、145 名のスギ花粉症患者血清中、134 名 (92.4%) の血清がCry j I 及びCry j IIと反応し、6 名 (4.1%) の血清がCry j I とのみに反応し、5 名 (3.4%) の血清がCry j IIとのみ反応することが判明した (1993年第43回日本アレルギー学会、橋本ら、日獣大、予研、国立相模原病院、林原生化研) 。つまり、スギ花粉症の発症には、Cry j I 及びCry j IIのどちらも重要であることが示された。

【００１１】Cry j I については、それをコードするcDNAがクローニングされ、その推定アミノ酸配列に基づき、T 細胞エピトープを含むペプチドが同定されている (WO94/01560、"ALLERGENIC PROTEINS AND PEPTIDES FROM JAPANESE CEDAR POLLEN")。Cry j IIについては、N 末端のアミノ酸配列のAla 、Ile 、Asn 、Ile 、Phe 、Asn 、Val 、Glu 、Lys 及びTyr の10アミノ酸残基が報告されている (Sakaguchi, M., et al., Allergy 45, 309-312, 1990) に過ぎない。

【００１２】

【発明が解決しようとする課題】本発明は、スギ花粉症の診断、予防及び治療に有用な、スギ花粉アレルゲンCry j IIの少なくとも１つのエピトープ、特にT 細胞エピトープを含むタンパク質またはペプチド、または該タンパク質またはペプチドをコードするＤＮＡを提供することを目的とする。

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【００１３】

【課題を解決するための手段】本願発明者らは、上記課題を解決するために、

(1)Cry j II の全アミノ酸配列 (一次構造) の解明

(2)Cry j II の全アミノ酸配列をカバーするオーバーラップペプチドの作製

(3)Cry j II アレルゲンを特異的に認識するT 細胞ラインを個人別に樹立

(4) 抗原提示細胞 (B 細胞株) の樹立

(5)T細胞エピトープを含むオーバーラップペプチドの同定

を行い、本発明を完成した。なお、本発明でいうエピトープは、T 細胞エピトープに限られるものではないが、以下T 細胞エピトープについて詳述する。これらの各ステップを以下に説明する。

【００１４】(1)Cry j II の全アミノ酸配列の解明

① cDNAのクローニング

a. RNAの抽出

RNA を抽出する際、通常、初期段階で蛋白質を除去する。このため一般的な方法として、フェノール抽出方法、グアニジウム塩、界面活性剤、尿素などの蛋白質変性剤などを用いる方法がある。

【００１５】スギ花粉からのRNA 抽出は、Breiteneder ら (Int. Arch. Allergy Appl. Immunol. 87: 19-24, 1988) の方法に改良を加えて行うことができる。

【００１６】スギ花粉を、10～20倍量の抽出緩衝液 (100mM LiCl、10mMNa_2 ＥＤＴＡ、1%SDS 、20% メルカプトエタノール、100mM Tris-HCl pH 9.0) に懸濁し、これに等量のフェノールとクロロホルムの混液 (フェノール：クロロホルム：イソアミルアルコール＝24：24：1) を加えホモジェナイズする。次いで遠心 (10,000g 、10～15分) し、フェノール・クロロホルム層と、水層の二層に分離する。このとき変性した蛋白質はフェノール・クロロホルム層に、核酸は水層に移行する。水層にフェノール・クロロホルム混液を加え、振盪し水層に残存している蛋白質などの不純物をフェノール・クロロホルム層に移行させ除去する。このような操作を２回繰り返す。

【００１７】得られた水層からRNA を抽出するには、高濃度のLiCl(2～4M) またはCH_3 COONa(3M)が存在するとDNA 及び蛋白質は上清に残り、tRNA以外のRNA は沈殿する性質を利用する。水層に同量の2 ～4MのLiClを添加し、RNA を沈殿させる。次いでこの水層を水に溶解し、0.1 ～0.3 容の冷エタノール (-20 ℃) を加え、RNA を沈殿させる (エタノール沈殿) 。次いで遠心 (10,000g 、30分) して沈殿を回収し、水に溶解して全RNA 分画を得る。

【００１８】b. mRNA の調製とcDNAの合成

Cry j IIのmRNAは、3'末端にポリ(A) 鎖を持つので、こ

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れと相補するリガンドとして12～18塩基のデオキシチミジン(dT)を結合したオリゴdTセルロースカラム(Clontech Laboratories Inc. 社製、CA、USA)にmRNAを吸着される。スギ花粉RNA に緩衝液(3M NaCl、1mM EDTA、10mM Tris-HCl 、pH7.4)を加えてmRNAをカラムに吸着させる。mRNAは、ベッド体積の2 ～3 倍量のNaClを含まない緩衝液(1mM EDTA 、10mM Tris-HCl 、pH7.4)で溶出する。

【００１９】得られたmRNAからのcDNAライブラリーの作製は、現在市販されているファージをベクターに用いたcDNAライブラリー作製キット(Amersham International plc. 社製、Buckinghamsbare 、England)を用いて行うことが出来る。

【００２０】c. Cry j II cDNAのスクリーニング

Cry j IIのN 末端アミノ酸10残基が既に判明しているが、このアミノ酸配列から推定した塩基配列を持つ合成DNA をプローブとして、Cry j II cDNA をスクリーニングすることができる。プローブに用いるDNA を合成する場合、可能性のあるコドンを含むオリゴヌクレオチドを全て合成するよりも、可能性のある複数のコドン配列に対してハイブリダイズするようなオリゴヌクレオチドを設計することが望ましい。この合成オリゴヌクレオチドプローブの5' 末端を[γ- ^{32}P]ATPとポリヌクレオチドキナーゼによって標識し、プラークハイブリダイゼーション法により、前記cDNAライブラリーから陽性クローンをスクリーニングする。

【００２１】得られた陽性クローンよりファージDNA を調製し、挿入cDNA断片を分離し、pUC18 等のプラスミドにサブクローンする。必要に応じてオリゴヌクレオチドプライマーを合成し、Sanger法等により塩基配列を決定し、クローンを同定する。本発明者らが単離したCry j II cDNA の全長の塩基配列を、配列番号５に示す。

【００２２】Cry j IIをコードするcDNAは、全体で1733 bpからなり、翻訳開始と想定されるコドン（45～47位のヌクレオチドATG ）から終止コドン（1587～1589位のヌクレオチドTAA ）に至るオープンリーディングフレームを含み、514 アミノ酸をコードしている。オープンリーディングフレーム部分の塩基配列を配列番号３に示し、また該塩基配列がコードするアミノ酸配列を配列番号１に示す。配列番号３で示される塩基配列には、個体間での対立遺伝子変異による多型性 (polymorphism) 及びその結果としてのアミノ酸配列の変異が考えられるがそのような変異を有するCry j IIの塩基配列及びアミノ酸配列も本発明に包含される。207 ～236 位のDNA 配列のコードするアミノ酸配列はAla 、Ile 、Asn 、Ile 、Phe 、Asn 、Val 、Glu 、Lys 、Tyr であり、成熟型Cry j IIのN 末端アミノ酸配列 (Sakaguchi, M., et al., Allergy 45, 309-312, 1990) と一致する。N 末端の54アミノ酸は、他のシグナルペプチドに見られる疎水性アミノ酸に富み、また成熟型Cryj IIに含まれていないことからシグナルペプチドと考えられる。

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【００２３】207 位から終止コドン1587～1589位までのDNA 配列がコードするCry j IIは、N 末端のAla からC 末端のPro まで460 個のアミノ酸残基からなり、成熟型Cryj IIと考えられる。該成熟型Cry j IIに対応する塩基配列を配列番号４に、該塩基配列にコードされるアミノ酸配列を配列番号２に示す。配列番号２に示すアミノ酸配列からなるCry j IIの理論上の分子量は50,444Daである。一方、天然の成熟型Cry j IIは、還元条件下のSDS-ポリアクリルアミド電気泳動 (SDS-polyacrylamide gel electrophoresis) で45KDa の位置にそのバンドが現れる (Sakaguchi, M., et al., Allergy 45, 309-312, 1990) 。このことから、Cry j IIのC 末端はプロセッシングを受けているものと考えられる。また成熟Cry j IIのアミノ酸配列の中には、N-グリコシド結合の可能性のあるAsn-X-Ser/Thr が存在する。

【００２４】Cry j IIをコードするDNA の全長またはその一部を含むDNA は、蛍光標識、放射性標識あるいは酵素標識等によって標識することにより、生化学検査または関連蛋白質若しくは類似の配列を含む蛋白質をコードするDNA のスクリーニング等のためのプローブ、プライマーとして使用できる。また発現ベクターに接続して、少なくとも１つのエピトープを含むタンパク質またはペプチドを発現させることもできる。

【００２５】②組換えCry j II （rCry j II ）の発現

rCry j II または少なくとも一つのCry j IIのエピトープを含む組換えタンパク質またはペプチドは、それぞれをコードするcDNAを発現ベクターに組込み、大腸菌、昆虫細胞、酵母または哺乳動物に導入し、培養することにより得ることができる。しかし、大腸菌などの原核細胞を使う発現系は、適切な糖鎖の付加(glycosylation) が行われないために、rCry j II の発現には酵母などの真核細胞を使用することが好ましい場合がある。

【００２６】Cry j IIの幾つかの発現システムの例を以下に示す。

【００２７】a.　大腸菌での発現

T7ファージのプロモーターとRNA ポリメラーゼを用いる系(F. W. Studier, A.H. Rosenberg, J. J. Dunn, J. W. Dubendonff, "Methods in Enzymology", ed. by D. D. V. Goeddel, vol. 185, p. 60, Academic Press, New York, 1990)は、極めて発現の成功率が高いので、本発明に好適に使用できる。この系は、T7ファージのポリメラーゼ遺伝子を持つ大腸菌宿主BL21(DE3) に、T7ファージプロモーターの下流のマルチクローニングサイトに目的の遺伝子を挿入した組換えプラスミドを導入して、IPTG存在下で、目的の遺伝子を発現させるシステムである。例えば発現ベクターとしてpGEMEX-1 (Promega 社) などが使用できる。

【００２８】また、目的の蛋白質を、大量発現可能な蛋白質と融合させて発現させる系が市販されており、これらの系は精製にアフィニティーカラムが使え、精製効率

がよく、本発明に好適に使用できる。例えば、融合蛋白質にβ- ガラクトシダーゼを有する発現ベクターpUEX(Amersham)を用いると、rCry j II はβ- ガラクトシダーゼとの融合蛋白質として得られ、アフィニティカラムで効率よく精製することが出来る。また、グルタチオンS-トランスフェラーゼを有するpGEX(Pharmacia)や、マルトース結合蛋白質を用いたpMAL(New England Biolabs, Berverly, MA)などは、その融合部位に血液凝固因子Xaの切断部位が導入されており、Cry j IIを分離することができる。

【００２９】b. 酵母での発現

酵母を宿主とする系は発現産物のグリコシレーションが可能であり、このことは糖蛋白質であるCry j IIの発現に好都合である。例えば酵母による異種蛋白質の発現系としては、ピキア酵母を宿主として用いる方法が知られており（特開昭61-108383 号公報、特開昭61-173781 号公報、特開昭63-44899号公報、特開平1-128790号公報等）、本発明に好適に使用できる。その他の酵母による発現系については、D. Emr Scott, "Methods in Enzymology", ed. by D. V. Goeddel, vol. 185, p.231, Academic Press, New York (1990) に詳述されており、本発明で使用できる。

【００３０】c. 昆虫細胞での発現

昆虫細胞中を宿主とする系は発現産物のグリコシレーションが可能である。バキュロウイルスを用いた外来遺伝子発現システムは市販されており(PharMingen, San Diego, CA, USA)、本発明に好適に使用できる。このシステムについては、Luckow, V. A. らの Trends in the Development of Baculovirus Expression Vector, Bio/Technology (1987年9 月11日) に記載されている。

【００３１】d. 哺乳動物細胞での発現

哺乳類プロモーター（例えばメタロチオネイン）、ウイルスプロモーター（例えばSV40初期プロモーター）等を持つ発現ベクターに組み込み、哺乳動物細胞に導入することにより高発現させることができる。

【００３２】(2) オーバーラップペプチドの合成

花粉症患者のT 細胞が認識するCry j IIのT 細胞エピトープを分子レベルで解明するために、配列番号２に記載のCry j II cDNA のコードするアミノ酸配列に基づき、N 末端のAla からC 末端のPro に至る全460 アミノ酸残基をカバーするオーバーラップペプチドを作製する。これらのオーバーラップペプチドは、市販されているペプチド自動合成装置により容易に合成することができる。これらのオーバーラップペプチドの中から、少なくとも一つのエピトープを含むペプチドを同定する。特に、T 細胞エピトープを同定するためには、花粉症患者の末梢血リンパ球から、Cry j IIを特異的に認識し増殖応答するT 細胞ラインを樹立する必要がある。一般に、患者毎に反応するT 細胞エピトープが異なるので、患者毎にT 細胞ラインを樹立することが望ましい。

【００３３】(3)T細胞ラインの樹立

Cry j II抗原特異的なT 細胞ラインを樹立するには、通常患者の末梢血リンパ球をCry j II抗原の存在下、7 日間程度培養して抗原刺激によりT 細胞を活性化し、さらに、活性化T 細胞を、抗原と抗原提示細胞と共に7 日間培養することを数回繰り返して抗原刺激することにより、抗原特異的T 細胞ラインを作製することができる。しかしながら、T 細胞が増殖因子のIL-2の存在下でよく増殖している場合は、抗原刺激は最初だけにすることが望ましい。T 細胞ラインを数度抗原刺激すると、増殖率の高いT 細胞が選択的に取れ、T 細胞エピトープを含むペプチドを同定する場合において、エピトープによっては十分な増殖応答を示さない場合が生じる。

【００３４】使用する抗原としては、原理的には天然型Cry j II抗原が望ましいが、極微量しかスギ花粉から抽出できないことから、組換えCry j II（rCry j II）あるいはオーバーラップペプチドの混合物も好適に使用できる。rCry j II は、大腸菌で発現させ精製したものが利用できる。

【００３５】(4) 抗原提示細胞（B 細胞株）の樹立

抗原提示細胞としては、T 細胞ラインと同一人の末梢血リンパ球を、マイトマイシンC 処理あるいは放射線照射して増殖能力を失わせたものが望ましい。しかし、採血回数が多くなるため、Epstein-Barr virus (EBV) を自己のB リンパ球に感染させトランスフォーメーションを起こさせたものは、in vitroで増殖し続けリンパ芽球様細胞株（B 細胞株）となるので、このB 細胞株を抗原提示細胞として用いてもよい。B 細胞株の樹立方法は既に確立されている［組織培養の技術第二版、187-191 頁、日本組織学会編(1988.8.10)］。

【００３６】(5)T細胞エピトープを含むオーバーラップペプチドの同定

それぞれの患者固有のT 細胞ラインが認識する、T 細胞エピトープを含むペプチドは以下のようにして同定される。ここで「認識する」という意味は、T 細胞レセプターが抗原エピトープ（MHC 分子を含めて）と特異的に結合し、その結果、T 細胞が活性化されることを意味し、活性化の状態は、リンホカインの産生や、DNA の合成を［^{3}H］チミジンの取込み量を指標として測定することにより観察される。すなわち、T 細胞ラインとマイトマイシンC 処理した同一人のB 細胞株とを、96穴平底プレートに播種し、オーバーラップペプチドと共に混合培養し、［^{3}H］チミジンの取込み量（cpm）を液体シンチレーションカウンターで測定する。その際、［^{3}H］チミジンの取込みは、個々の培養系で異なるため、例えば、個々のペプチドに対するT 細胞ラインの［^{3}H］チミジン取込み量（cpm）を、抗原を添加していないコントロールの［^{3}H］チミジン取込み量（cpm）で除した数（stimulation index: SI）が2 以上をT 細胞エピトープを含むペプチドと同定する。同定されたT 細胞エピ

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トープを含むペプチドは、図４に列挙されている。

【００３７】このようにして得られた本発明のCry j IIの少なくとも一つのT 細胞エピトープを含むペプチドについては以下のことが考えられる。HLA クラスII分子と結合して抗原提示されるペプチドの長さは、ペプチドの解析結果（Chicz, R. M. etal.: J. Exp. Med., 178: 27-47, 1993 ）から、およそ10～34のアミノ酸残基からなるものと考えられるので、本発明のT 細胞エピトープを含むペプチドはこのような長さのペプチドも含まれる。また、本発明のペプチドにアミノ酸置換、欠失あるいは付加などの修飾を行い、これらの修飾ペプチドに対する患者毎のT 細胞ラインの増殖応答を測定することによって、本発明のCry j IIの少なくとも一つのT 細胞エピトープを含むペプチドと免疫学的に同機能を有する修飾ペプチドを容易に作製することは、当業者が容易に実施しうることであるので、これらの修飾ペプチドも本発明に包含される。

【００３８】現在、減感作療法で使用されている減感作剤はスギ花粉から抽出された粗抗原であり、多量の多糖類を含んでいる。ロット差がかなりあり、一旦減感作療法を開始した後、ロットを変えるとアナフィラキシーを起こすことが稀にある。また、減感作の治療効果も、減感作治療が開始されて以来余り改善されておらず、減感作療法で著効と診断されるのは約30% の患者である。

【００３９】本発明のT 細胞のエピトープを含むペプチドのうち、花粉症患者の半分以上のT 細胞ラインと反応する各々のペプチドは、これらの各ペプチドを単独もしくはいくつかを混合したペプチドを用いて減感作療法を行った場合には、治療した患者の半分以上で減感作が行える可能性がある。また、使用するペプチドは、化学的に合成されたペプチドであるため、アナフィラキシーのような副作用を生じる可能性は低くなると考えられる。例えば、図５は、18名の花粉症患者から樹立されたT 細胞ラインがそれぞれ認識するオーバーラップペプチドを、重要度指数［「平均刺激係数」（「オーバーラップペプチド刺激によるT 細胞ラインの［^{3}H］チミジン取込み量(cpm)」を「抗原を添加しない場合の［^{3}H］チミジン取込み量(cpm)」で割った値の平均値）と「出現頻度(%)」（「試験した全T 細胞ライン」に対する「被験ペプチドをT 細胞エピトープとして認識したT 細胞ライン」の割合(%)）とを乗じた値］で示したものであるが、図中の番号14、17、29、38、48、68、70および71のペプチドは平均刺激係数が約3.9 以上である上、重要度指数が200 を超えており、減感作治療に特に有効であると考えられる。

【００４０】なお、本発明者が明らかにし、図５に示された少なくとも一つのT 細胞エピトープを含むペプチドの中には、後述のB 細胞エピトープを含むことが判明した２種類のペプチドと共通部分を有するペプチドは含まれていない。従って本発明のペプチドは、B 細胞エピトープを刺激しないと考えられるので、減感作剤として実用化可能であると考えられる。

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【００４１】また、本発明のT 細胞のエピトープを含むペプチドを経口投与して、経口免疫寛容を行うことも可能と考えられる。経口免疫寛容（経口減感作）は現在開発中の治療法であるが、効果を示す結果が報告され始めている。例えば、Myelin Basic ProteinのT 細胞エピトープ（ペプチド配列21-40 、71-90 ）をマウスに経口投与すると「Experimental Autoimmune Encephalomyelitis （略してEAE ）発症」を抑制したことが報告されている［上野川修一、久恒辰博、八村敏志、経口免疫寛容の分子生物学、蛋白質核酸酵素、39、2090-2101 （記載頁2098右、9-24行）1994年］。これらの例から、スギ花粉症においても、同定したT 細胞エピトープペプチドをそのまま経口投与するか、あるいは胃で消化されないように何らかのカプセルに封入する等の工夫を行って経口投与すれば、免疫寛容状態になる可能性がある。スギ花粉飛散時期の前、具体的には12～1 月期に経口的にエピトープペプチドを投与し、免疫寛容状態を誘導しておく。この状態だとスギ花粉が飛散して鼻粘膜に花粉が付着しても、症状が出ないか、あるいは症状が軽くなることが期待される。

【００４２】さらにまた、本発明のT 細胞のエピトープを含むペプチドに、アミノ酸置換、欠失あるいは付加などの修飾を加えたアナログペプチドを合成し、HLA クラスII分子には結合するが、T 細胞には情報が伝わらないアナログペプチドを同定する。これらのペプチドは、例えば点鼻薬として患者に使用すれば、天然のT 細胞エピトープを競合的に阻害するので、発症予防が期待される。

【００４３】なお、本発明でいうエピトープには、B細胞エピトープも含まれる。B細胞エピトープの同定は、オーバーラップペプチドと患者血清IgE 抗体との反応性の測定、オーバーラップペプチドによる患者血清と抗原との結合の阻害の検出等の公知の方法によって行うことができる（特開平６－６９３３６号参照）。既に、１価のB 細胞エピトープは、アレルギー反応の抑制に有用であることが知られている。これは、１価のB 細胞エピトープは、肥満細胞または好塩基球上の対応するIgE 分子と結合し、多価エピトープによるIgE 分子架橋の形成を阻害することによるものと考えられている。本発明者らは、本発明のCry j IIの全アミノ酸配列をカバーするオーバーラップペプチドを合成し、これらのペプチドとスギ花粉症患者血清IgE 抗体との反応を酵素抗体法で測定した結果、ペプチド「Gln Cys Lys Trp Val Asn Gly Arg Glu Ile Cys （アミノ酸配列113 ～123 ）」および「Cys Thr Ser Ala Ser Ala Cys Gln Asn （アミノ酸配列293 ～301 ）」はB 細胞エピトープを含んでいることを明らかにした。このようなCry j IIのB 細胞エピトープを含むペプチドは、スギ花粉症の診断、予防及び治療

に有用である。

【００４４】

【実施例】以下本発明を実施例に基づいて詳細に説明するが、本発明はこれに限定されない。

【００４５】＜スギ花粉の採取＞スギ花粉は静岡県及び神奈川県内で2 月に伐採されたスギの枝に着花した雄花から採取した。Cryj II 抗原性精製用のスギ花粉は-70 ℃で保存し、RNA 調製用のスギ花粉は液体窒素中で急速凍結した後、-70 ℃で保存した。

【００４６】＜RNA の抽出＞Breiteneder ら(Int. Arch. Allergy Appl. Immunol. 87:19-24 1988) の方法を基にして改良を加えることによりスギ花粉からRNA を抽出した。

【００４７】凍結保存したスギ花粉1gを氷冷した15mlの抽出緩衝液(100mM LiCl 、10mMNa_2EDTA、1%SDS 、20% 2-メルカプトエタノール、100mM Tris-HCl、pH 9.0)に懸濁し、さらに、15mlのフェノール：クロロフォルム：イソアミルアルコール(24:24:1) を添加した。この懸濁液をテフロンホモジェナイザーに移し、テフロンペステルをモーターで最高回転で回しながら、20～30ストロークホモジェナイズした。この後、遠心操作(10,000g、15分) で水層と有機層に分離して水層を得た。水層に同量のフェノール：クロロフォルム：イソアミルアルコールを加え、5 分間振蕩の後、遠心分離(10,000g、15分) で水層を得た。同様の操作を2 回繰り返し、さらに15mlのクロロフォルム：イソアミルアルコール(24:1)を用いて1 回行った。得られた水層に同量の4M LiCl を添加して-20 ℃で一晩放置した。凍結した溶液を室温で溶解し、遠心操作(20,000g、30分) で沈澱を得た。この沈澱を少量の滅菌蒸留水に溶解し、0.3 容の3M CH_3COONa 、pH 5.2と2.5 容のエタノールを加え、-20 ℃で60分間放置した。遠心操作(10,000g、30分) により回収した沈渣を滅菌蒸留水に再溶解して全RNA 分画とした。

【００４８】＜スギ花粉mRNAの調製とcDNAの合成＞スギ花粉全RNA1mgを出発材料として同量の結合緩衝液(3M NaCl、1mM EDTA、10mM Tris-HCl 、pH 7.4) を添加した後、オリゴdTセルロースを事前にパックしたスパンカラム(CLONETECH Laboratories Inc.社製、CA、USA)に吸着させ、溶出緩衝液(1mM EDTA 、10mM Tris-HCl 、pH 7.4) で溶出することにより約10μg のmRNAを精製した(CLONETECH Lab. Inc.社添付プロトコールに従った) 。続いて、精製mRNA 5μg からcDNA合成システムプラス(Amersham International plc.社製、Buckinghamshare 、England)を使用し、添付されているプロトコールに従ってcDNA約4 μg を合成した。

【００４９】＜オリゴヌクレオチドプローブの合成＞Cry j IIのN 末端から10残基のアミノ酸配列を図１Ａに示す。このアミノ酸配列から予想されるcDNAの配列は図１Ｂである。オリゴヌクレオチドプローブ (Oligo CJII) としてその配列に相補的に、また４カ所で２種類の塩基を用いているので、合計16種類の混合物として合成した（図１Ｃ）。混合物として種類を減らすためにG:T 塩基対を許容している。

【００５０】＜Cry j II cDNA のクローニング＞cDNAライブラリーの作製はcDNAクローニングシステムλgt10 (Amersham International plc. 社製、Buckinghamshare 、England) を使用し、添付されているプロトコールに従って行った。上述のcDNA 1μg をλgt10に組み込みcDNAライブラリーを作製した。約50万のライブラリーのうち約5,000 のクローンを直径150mm のプレート１枚にまいた。スクリーニングのためのプローブは上記のオリゴヌクレオチド (Oligo CJII) をT4 polynucleotide kinaseにより[γ-^{32}P]ATP (7,000Ci/mmol ICN Biochemicals, Inc. 社製) で標識して用いた。ファージDNAを固定化したニトロセルロースフィルターを5 ×SSPE (1 ×SSPE:0.18M NaCl 、10mMリン酸ナトリウム、1mM EDTA) 、5 ×FBP (1 ×FBP:0.02% Ficoll、0.02%牛血清アルブミン、0.02% ポリビニルピロリドン) 、0.3%SDS 、100 μg/ml tRNA を含む溶液に48℃1 時間以上浸すことによりプレハイブリダイズした。この後ニトロセルロースフィルターを新たに調製した同溶液に浸し、^{32}P ラベルしたプローブ (Oligo CJII) を加えて48℃で一晩ハイブリダイゼイションを行った。この後フィルターを6 ×SSC (1 ×SSC:0.15M NaCl、0.015Mクエン酸ナトリウム) と0.1%SDS を含む溶液で室温30℃、48℃5 分洗浄した後、オートラジオグラフィーを行った。4 個の強いシグナルが検出され、そのうちの１つのファージDNA を抽出し、制限酵素EcoRI で切断したところ約1.7KbpのDNA断片が挿入されていることが判明した。挿入断片をpUC118にサブクローニングし、キロシークエンスデレーションキット（宝酒造社製）を用いてデレーションミュータントを作製し全塩基配列の決定に用いた。塩基配列は合成プライマーと色素標識ジデオキシターミネイターを用いてプライマー伸長反応を行い、自動シークエンサー（モデル370A、Applied Biosystems、Japan ）で判読することにより決定した。決定されたcDNA全塩基配列を配列番号５に示す。また、オープンリーディングフレームのみの塩基配列を配列番号３に（該塩基配列がコードするアミノ酸配列を配列番号１に）、成熟Cry j IIをコードする塩基配列を配列番号４に（該塩基配列がコードするアミノ酸配列を配列番号２に）示す。

【００５１】＜組換えCry j IIの大腸菌での発現＞Promega 社より市販されている大腸菌発現ベクターpGEMEX-1はT7プロモーター、T7 gene10 のコーディングシークエンスおよびT7ターミネーターをもち、オープンリーディングフレームをT7 gene10 の下流のマルチクローニングサイトに挿入してT7 RNAポリメラーゼを発現する大腸菌（例BL21(DE3) ）に導入することにより高発現を行うベクターである。Cry j II cDNA をBamHI （cDNAの両端に連結したアダプターはBamHI サイトを含む）で消化して

cDNAフラグメントを切り出しpGEMEX-1のBamHI サイトに組み込みCry j IIの発現ベクターpEXCJII を構築した。pEXCIIはT7 gene10 発現産物（23kD）とCry j II蛋白質（50kD）との融合蛋白質（T7 Cry j II 、73kD）を発現し得る。pEXCIIを大腸菌BL21(DE3）に導入した形質転換体を培養しIPTGでT7 RNAポリメラーゼを誘導してCry j IIの発現を行った。発現した大腸菌の細胞抽出液をSDSポリアクリルアミドゲル電気泳動にかけた。pEXCIIを保持するBL21(DE3）には、約73kDのT7 Cry j II と思われるバンドが見られた。しかし、対照のpGEMEX-1を保持するBL21(DE3）または親株BL21(DE3）には、これらのバンドは見られなかった。

【００５２】＜Cry j IIとT7 gene10 との融合タンパク質（T7Cry j II）のスギ花粉症患者血清との反応性＞T7 Cry j II を発現した大腸菌の抽出液を、SDS ポリアクリルアミドゲル電気泳動した後、Millipore 社製PVDF膜にウェスタンブロッティング（Western Blotting）し、スギ花粉症患者5 人、健常人3 人の血清との反応性を検討した。対照としてpGEMEX-1を保持するBL21およびT7 gene10 とCry j I との融合蛋白質（T7Cry j I）を発現したBL21の抽出液、スギ花粉より精製した天然型Cry j I を同時にブロットして反応を調べた。図２に示すように、2 人の患者血清がT7 Cry jII と反応した。2 人の患者血清ともT7 Cry j II 、天然型 Cry j Iには反応しているがpGEMEX-1を保持するBL21抽出液およびT7 Cry j Iには反応していない。これらの結果からT7Cry j IIはスギ花粉症患者血清中のIgE と反応する抗原性を持っていることが確認された。

【００５３】＜オーバーラップペプチドの合成＞オーバーペプチドの合成は、Peptide Synthesizer PSSM-8（島津製作所製）を用いて行なった。配列番号２に示すCry j IIの一次構造を基にして、N 末端側55番目のAla から始まり、C 末端のPro まで、10残基のオーバーラップ部分を含む15量体のオーバーラップペプチド90種類を合成した。図３～６にアミノ酸の１文字コードを用いて、合成した全てのオーバーラップペプチドを示す。

【００５４】＜B 細胞株の樹立＞Ficoll-Paque比重遠心法で得た末梢血リンパ球（1 ×10^6 ）を、約1 ×10^6 PFU （plaque forming units）のEpstein-Barr virus (EBV ）と共に37℃で1 時間インキュベートし、ウイルスを細胞に感染させた。このウイルス感染細胞を24ウェル培養プレートに移し、100ng/mlのサイクロスポリンA の存在下で2 週間前後培養すると、B 細胞コロニーが出現してくる。この時点で半分に分け、新しいウェルに植え継いだ。順次この操作を繰り返して継代培養を行っていくと、自己増殖可能なB 細胞が出現してくる場合がある。この自己増殖B 細胞を含むウェルの細胞をイクスパンド（expand）し、増殖を確認した後、25cm^2 培養フラスコに移して更に30～50日間培養を行い、EBV によってトランスフォームされた（EBV-transformed ）B 細胞株を得た。B 細胞株の一部は凍結保存した。

【００５５】＜Cry j II抗原特異的T 細胞ラインの樹立＞スギ花粉症患者18名末梢血からリンパ球を通常用いられているFicoll-paque比重遠心法で単離し、使用するまで液体窒素中に保存した。スギ花粉症患者の末梢血リンパ球（4 ×10^6 個）を、2 mlの自己の血漿20% を添加したRPMI-1640 に懸濁し、10μg/mlの大腸菌で発現させ精製した組換えCry j II抗原と共に24穴培養プレート上で7-8 日間培養した。Cry j II抗原刺激を受けて活性化された（幼弱化反応、blastogenesis ）T 細胞が顕微鏡下で確認できた時点で5 Unit/ml のIL-2を添加し、一晩培養した。翌日からは、20 Unit/ml IL-2 、20% ヒトAB型血清（市販品）を添加したRPMI-1640 で毎日培養液を代えながら、9 日間培養した。この時点で、Cry j II抗原を特異的に認識する増殖したT 細胞ラインの一部を凍結保存した。さらにT 細胞ラインを上記培養液中で4 日間培養し、エピトープの同定用の細胞とした。

【００５６】＜T 細胞エピトープを含むオーバーペプチドの同定＞18名の花粉症患者から樹立したT 細胞ラインについてそれぞれスギ花粉アレルゲンオーバーラップペプチドとともに培養し、Cry j II抗原特異的T 細胞エピトープを含むペプチドの同定を行った。

【００５７】T 細胞ラインと同一の患者から樹立した培養B 細胞株を50μg/mlのマイトマイシンC で30分間処理し、細胞をRPMI-1640 で4 回洗浄した。このB 細胞を96穴平底プレート（96-well flat-bottomed plate ）に播種（5 ×10^4 /well ）した後、Cry j II（25μg/ml最終濃度）あるいは各オーバーラップペプチド（最終濃度0.5 μM ）を各々のウェルに添加し、約60～90分間培養した。T 細胞ライン（2×10^4 /well ）を各ウェルに播種し、48時間培養の後、0.5 μl/Ci[^{3} H]チミジンをウェルに添加し、さらに16時間培養した。細胞を細胞ハーベスターを用いてガラスフィルター上に捕集し、乾燥してから、細胞内に取込まれた[^{3} H]チミジンのカウント（cpm ）を液体シンチレーションカウンターで測定した。

【００５８】測定はtriplicate cultureで行い、結果は、オーバーラップペプチド刺激によるT 細胞ラインの[^{3} H]チミジン取込み量（cpm ）を、抗原を添加しない場合（コントロール）の[^{3} H]チミジン取込み量（cpm ）で割った値である刺激係数（stimulation index; SI ）で算出し、SIが2 以上の値を示したオーバーラップペプチドを、T 細胞エピトープを含むオーバーペプチドと同定した。図７及び図８は、18名の花粉症患者からそれぞれ樹立されたCry j II抗原特異的T 細胞ラインの少なくとも１種類が認識する少なくとも一つのT 細胞エピトープを含むペプチドを示す。また、図９は、全てのオーバーラップペプチドの「平均刺激係数」（複数の実験によって得られた刺激係数の平均値）「出現頻度(%) 」「重要度指数」を示している。

【００５９】
【発明の効果】本発明のCry j IIの少なくとも一つのエピトープを含むペプチド、T 細胞エピトープを含むペプチドは、スギ花粉症の診断、予防及び治療に有用である。
【００６０】さらにまた、HLA クラス　分子には結合するが、T 細胞には情報が伝わらないようなアナログペプチドを合成し、これらのペプチドを競争阻害によるスギ花粉症発症予防に用いることも可能である。
【００６１】
【配列表】
配列番号：1
配列の長さ：514
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：タンパク質
配列：

Met Ala Met Lys Leu Ile Ala Pro Met Ala Phe Leu Ala Met Gln Leu
5 10 15
Ile Ile Met Ala Ala Ala Glu Asp Gln Ser Ala Gln Ile Met Leu Asp
20 25 30
Ser Val Val Glu Lys Tyr Leu Arg Ser Asn Arg Ser Leu Arg Lys Val
35 40 45
Glu His Ser Arg His Asp Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr
50 55 60
Gly Ala Val Gly Asp Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr
65 70 75 80
Ala Trp Gln Ala Ala Cys Lys Asn Pro Ser Ala Met Leu Leu Val Pro
85 90 95
Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys
100 105 110
Gln Pro His Phe Thr Phe Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln
115 120 125
Asn Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys
130 135 140
Leu Thr Gly Phe Thr Leu Met Gly Lys Gly Val Ile Asp Gly Gln Gly
145 150 155 160
Lys Gln Trp Trp Ala Gly Gln Cys Lys Trp Val Asn Gly Arg Glu Ile
165 170 175
Cys Asn Asp Arg Asp Arg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr
180 185 190
Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His
195 200 205
Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile
210 215 220
Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp Gly Ile Asp Ile Phe Ala
225 230 235 240
Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly Asp Asp
245 250 255
Cys Val Ala Ile Gly Thr Gly Ser Ser Asn Ile Val Ile Glu Asp Leu
260 265 270
Ile Cys Gly Pro Gly His Gly Ile Ser Ile Gly Ser Leu Gly Arg Glu
275 280 285
Asn Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe
290 295 300
Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser
305 310 315 320
Gly Met Ala Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile Asn Ser

325 330 335
Glu Asn Pro Ile Leu Ile Asn Gln Phe Tyr Cys Thr Ser Ala Ser Ala
340 345 350
Cys Gln Asn Gln Arg Ser Ala Val Gln Ile Gln Asp Val Thr Tyr Lys
355 360 365
Asn Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys
370 375 380
Ser Asp Ser Met Pro Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu
385 390 395 400
Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn
405 410 415
Gly Tyr Phe Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro
420 425 430
Ser Ala Lys Arg Lys Glu Ser Lys Ser His Lys His Pro Lys Thr Val
435 440 445
Met Val Glu Asn Met Arg Ala Tyr Asp Lys Gly Asn Arg Thr Arg Ile
450 455 460
Leu Leu Gly Ser Arg Pro Pro Asn Cys Thr Asn Lys Cys His Gly Cys
465 470 475 480
Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gln
485 490 495
Glu Tyr Tyr Pro Gln Arg Trp Ile Cys Ser Cys His Gly Lys Ile Tyr
500 505 510
His Pro

配列番号：2
配列の長さ：460
配列の型：アミノ酸
トポロジー：直鎖状
配列の種類：タンパク質

配列：
Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr Gly Ala Val Gly Asp Gly
5 10 15
Lys His Asp Cys Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala Ala Cys
20 25 30
Lys Asn Pro Ser Ala Met Leu Leu Val Pro Gly Ser Lys Lys Phe Val
35 40 45
Val Asn Asn Leu Phe Phe Asn Gly Pro Cys Gln Pro His Phe Thr Phe
50 55 60
Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys
65 70 75 80
Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu
85 90 95
Met Gly Lys Gly Val Ile Asp Gly Gln Gly Lys Gln Trp Trp Ala Gly
100 105 110
Gln Cys Lys Trp Val Asn Gly Arg Glu Ile Cys Asn Asp Arg Asp Arg
115 120 125
Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu Ile Ile Gln Gly
130 135 140
Leu Lys Leu Met Asn Ser Pro Glu Phe His Leu Val Phe Gly Asn Cys
145 150 155 160
Glu Gly Val Lys Ile Ile Gly Ile Ser Ile Thr Ala Pro Arg Asp Ser
165 170 175

21 22

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Pro Asn Thr Asp Gly Ile Asp Ile Phe Ala Ser Lys Asn Phe His Leu
            180                 185                 190
Gln Lys Asn Thr Ile Gly Thr Gly Asp Asp Cys Val Ala Ile Gly Thr
        195                 200                 205
Gly Ser Ser Asn Ile Val Ile Glu Asp Leu Ile Cys Gly Pro Gly His
    210                 215                 220
Gly Ile Ser Ile Gly Ser Leu Gly Arg Glu Asn Ser Arg Ala Glu Val
225                 230                 235                 240
Ser Tyr Val His Val Asn Gly Ala Lys Phe Ile Asp Thr Gln Asn Gly
                245                 250                 255
Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser Gly Met Ala Ser His Ile
            260                 265                 270
Ile Tyr Glu Asn Val Glu Met Ile Asn Ser Glu Asn Pro Ile Leu Ile
        275                 280                 285
Asn Gln Phe Tyr Cys Thr Ser Ala Ser Ala Cys Gln Asn Gln Arg Ser
    290                 295                 300
Ala Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser
305                 310                 315                 320
Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser Asp Ser Met Pro Cys
                325                 330                 335
Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser Gly Lys
            340                 345                 350
Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gly His
        355                 360                 365
Val Ile Pro Ala Cys Lys Asn Leu Ser Pro Ser Ala Lys Arg Lys Glu
    370                 375                 380
Ser Lys Ser His Lys His Pro Lys Thr Val Met Val Glu Asn Met Arg
385                 390                 395                 400
Ala Tyr Asp Lys Gly Asn Arg Thr Arg Ile Leu Leu Gly Ser Arg Pro
                405                 410                 415
Pro Asn Cys Thr Asn Lys Cys His Gly Cys Ser Pro Cys Lys Ala Lys
            420                 425                 430
Leu Val Ile Val His Arg Ile Met Pro Gln Glu Tyr Tyr Pro Gln Arg
        435                 440                 445
Trp Ile Cys Ser Cys His Gly Lys Ile Tyr His Pro
    450                 455                 460
```

配列番号：3
配列の長さ：1542
配列の型：核酸
トポロジー：直鎖状
配列の種類：cDNA to mRNA

配列：

```
ATGGCCATGA AATTAATTGC TCCAATGGCC TTTCTGGCCA TGCAATTGAT TATAATGGCG   60
GCAGCAGAAG ATCAATCTGC CCAAATTATG TTGGACAGTG TTGTCGAAAA ATATCTTAGA  120
TCGAATCGGA GTTTAAGAAA AGTTGAGCAT TCTCGTCATG ATGCTATCAA CATCTTCAAT  180
GTGGAAAAAT ATGGCGCAGT AGGCGATGGA AAGCATGATT GCACTGAGGC ATTTTCAACA  240
GCATGGCAAG CTGCATGCAA AAACCCATCA GCAATGTTGC TTGTGCCAGG CAGCAAGAAA  300
TTTGTTGTAA ACAATTTGTT CTTCAATGGG CCATGTCAAC CTCACTTTAC TTTTAAGGTA  360
GATGGGATAA TAGCTGCGTA CCAAAATCCA GCGAGCTGGA AGAATAATAG AATATGGTTG  420
CAGTTTGCTA AACTTACAGG TTTTACTCTA ATGGGTAAAG GTGTAATTGA TGGGCAAGGA  480
AAACAATGGT GGGCTGGCCA ATGTAAATGG GTCAATGGAC GAGAAATTTG CAACGATCGT  540
GATAGACCAA CAGCCATTAA ATTCGATTTT TCCACGGGTC TGATAATCCA AGGACTGAAA  600
```

23 24

CTAATGAACA GTCCCGAATT TCATTTAGTT TTTGGGAATT GTGAGGGAGT AAAAATCATC 660
GGCATTAGTA TTACGGCACC GAGAGACAGT CCTAACACTG ATGGAATTGA TATCTTTGCA 720
TCTAAAAACT TTCACTTACA AAAGAACACG ATAGGAACAG GGGATGACTG CGTCGCTATA 780
GGCACAGGGT CTTCTAATAT TGTGATTGAG GATCTGATTT GCGGTCCAGG CCATGGAATA 840
AGTATAGGAA GTCTTGGGAG GGAAAACTCT AGAGCAGAGG TTTCATACGT GCACGTAAAT 900
GGGGCTAAAT TCATAGACAC ACAAAATGGA TTAAGAATCA AAACATGGCA GGGTGGTTCA 960
GGCATGGCAA GCCATATAAT TTATGAGAAT GTTGAAATGA TAAATTCGGA GAACCCCATA 1020
TTAATAAATC AATTCTACTG CACTTCGGCT TCTGCTTGCC AAAACCAGAG GTCTGCGGTT 1080
CAAATCCAAG ATGTGACATA CAAGAACATA CGTGGGACAT CAGCAACAGC AGCAGCAATT 1140
CAACTTAAGT GTAGTGACAG TATGCCCTGC AAAGATATAA AGCTAAGTGA TATATCTTTG 1200
AAGCTTACCT CAGGGAAAAT TGCTTCCTGC CTTAATGATA ATGCAAATGG ATATTTCAGT 1260
GGACACGTCA TCCCTGCATG CAAGAATTTA AGTCCAAGTG CTAAGCGAAA AGAATCTAAA 1320
TCCCATAAAC ACCCAAAAAC TGTAATGGTT GAAAATATGC GAGCATATGA CAAGGGTAAC 1380
AGAACACGCA TATTGTTGGG GTCGAGGCCT CCGAATTGTA CAAACAAATG TCATGGTTGC 1440
AGTCCATGTA AGGCCAAGTT AGTTATTGTT CATCGTATTA TGCCGCAGGA GTATTATCCT 1500
CAGAGGTGGA TATGCAGCTG TCATGGCAAA ATCTACCATC CA 1542

配列番号：4
配列の長さ：1380
配列の型：核酸
＊トポロジー：直鎖状
配列の種類：cDNA to mRNA

＊

配列：

GCTATCAACA TCTTCAATGT GGAAAAATAT GGCGCAGTAG GCGATGGAAA GCATGATTGC 60
ACTGAGGCAT TTTCAACAGC ATGGCAAGCT GCATGCAAAA ACCCATCAGC AATGTTGCTT 120
GTGCCAGGCA GCAAGAAATT TGTTGTAAAC AATTTGTTCT TCAATGGGCC ATGTCAACCT 180
CACTTTACTT TTAAGGTAGA TGGGATAATA GCTGCGTACC AAAATCCAGC GAGCTGGAAG 240
AATAATAGAA TATGGTTGCA GTTTGCTAAA CTTACAGGTT TTACTCTAAT GGGTAAAGGT 300
GTAATTGATG GGCAAGGAAA ACAATGGTGG GCTGGCCAAT GTAAATGGGT CAATGGACGA 360
GAAATTTGCA ACGATCGTGA TAGACCAACA GCCATTAAAT TCGATTTTTC CACGGGTCTG 420
ATAATCCAAG GACTGAAACT AATGAACAGT CCCGAATTTC ATTTAGTTTT TGGGAATTGT 480
GAGGGAGTAA AAATCATCGG CATTAGTATT ACGGCACCGA GAGACAGTCC TAACACTGAT 540
GGAATTGATA TCTTTGCATC TAAAAACTTT CACTTACAAA AGAACACGAT AGGAACAGGG 600
GATGACTGCG TCGCTATAGG CACAGGGTCT TCTAATATTG TGATTGAGGA TCTGATTTGC 660
GGTCCAGGCC ATGGAATAAG TATAGGAAGT CTTGGGAGGG AAAACTCTAG AGCAGAGGTT 720
TCATACGTGC ACGTAAATGG GGCTAAATTC ATAGACACAC AAAATGGATT AAGAATCAAA 780
ACATGGCAGG GTGGTTCAGG CATGGCAAGC CATATAATTT ATGAGAATGT TGAAATGATA 840
AATTCGGAGA ACCCCATATT AATAAATCAA TTCTACTGCA CTTCGGCTTC TGCTTGCCAA 900
AACCAGAGGT CTGCGGTTCA AATCCAAGAT GTGACATACA AGAACATACG TGGGACATCA 960
GCAACAGCAG CAGCAATTCA ACTTAAGTGT AGTGACAGTA TGCCCTGCAA AGATATAAAG 1020
CTAAGTGATA TATCTTTGAA GCTTACCTCA GGGAAAATTG CTTCCTGCCT TAATGATAAT 1080
GCAAATGGAT ATTTCAGTGG ACACGTCATC CCTGCATGCA AGAATTTAAG TCCAAGTGCT 1140
AAGCGAAAAG AATCTAAATC CCATAAACAC CCAAAAACTG TAATGGTTGA AAATATGCGA 1200
GCATATGACA AGGGTAACAG AACACGCATA TTGTTGGGGT CGAGGCCTCC GAATTGTACA 1260
AACAAATGTC ATGGTTGCAG TCCATGTAAG GCCAAGTTAG TTATTGTTCA TCGTATTATG 1320
CCGCAGGAGT ATTATCCTCA GAGGTGGATA TGCAGCTGTC ATGGCAAAAT CTACCATCCA 1380

配列番号：5
配列の長さ：1733
配列の型：核酸
トポロジー：直鎖状
配列の種類：cDNA to mRNA

配列：

AGTTGAGTTC GAGACAAGTA TAGAAAGAAT TTTCTTTTAT TAAAATGGCC ATGAAATTAA 60
TTGCTCCAAT GGCCTTTCTG GCCATGCAAT TGATTATAAT GGCGGCAGCA GAAGATCAAT 120
CTGCCCAAAT TATGTTGGAC AGTGTTGTCG AAAAATATCT TAGATCGAAT CGGAGTTTAA 180

25 26

GAAAAGTTGA GCATTCTCGT CATGATGCTA TCAACATCTT CAATGTGGAA AAATATGGCG 240
CAGTAGGCGA TGGAAAGCAT GATTGCACTG AGGCATTTTC AACAGCATGG CAAGCTGCAT 300
GCAAAAACCC ATCAGCAATG TTGCTTGTGC CAGGCAGCAA GAAATTTGTT GTAAACAATT 360
TGTTCTTCAA TGGGCCATGT CAACCTCACT TTACTTTTAA GGTAGATGGG ATAATAGCTG 420
CGTACCAAAA TCCAGCGAGC TGGAAGAATA ATAGAATATG GTTGCAGTTT GCTAAACTTA 480
CAGGTTTTAC TCTAATGGGT AAAGGTGTAA TTGATGGGCA AGGAAAACAA TGGTGGGCTG 540
GCCAATGTAA ATGGGTCAAT GGACGAGAAA TTTGCAACGA TCGTGATAGA CCAACAGCCA 600
TTAAATTCGA TTTTTCCACG GGTCTGATAA TCCAAGGACT GAAACTAATG AACAGTCCCG 660
AATTTCATTT AGTTTTTGGG AATTGTGAGG GAGTAAAAAT CATCGGCATT AGTATTACGG 720
CACCGAGAGA CAGTCCTAAC ACTGATGGAA TTGATATCTT TGCATCTAAA AACTTTCACT 780
TACAAAAGAA CACGATAGGA ACAGGGGATG ACTGCGTCGC TATAGGCACA GGGTCTTCTA 840
ATATTGTGAT TGAGGATCTG ATTTGCGGTC CAGGCCATGG AATAAGTATA GGAAGTCTTG 900
GGAGGGAAAA CTCTAGAGCA GAGGTTTCAT ACGTGCACGT AAATGGGGCT AAATTCATAG 960
ACACACAAAA TGGATTAAGA ATCAAAACAT GGCAGGGTGG TTCAGGCATG GCAAGCCATA 1020
TAATTTATGA GAATGTTGAA ATGATAAATT CGGAGAACCC CATATTAATA AATCAATTCT 1080
ACTGCACTTC GGCTTCTGCT TGCCAAAACC AGAGGTCTGC GGTTCAAATC CAAGATGTGA 1140
CATACAAGAA CATACGTGGG ACATCAGCAA CAGCAGCAGC AATTCAACTT AAGTGTAGTG 1200
ACAGTATGCC CTGCAAAGAT ATAAAGCTAA GTGATATATC TTTGAAGCTT ACCTCAGGGA 1260
AAATTGCTTC CTGCCTTAAT GATAATGCAA ATGGATATTT CAGTGGACAC GTCATCCCTG 1320
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AAACTGTAAT GGTTGAAAAT ATGCGAGCAT ATGACAAGGG TAACAGAACA CGCATATTGT 1440
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AGTTAGTTAT TGTTCATCGT ATTATGCCGC AGGAGTATTA TCCTCAGAGG TGGATATGCA 1560
GCTGTCATGG CAAAATCTAC CATCCATAAT GAGATACATT GAAACTGTAT GTGCTAGTGA 1620
ATATTCTTGT GGTACAATAT TAGAACTGAT ATTGAAAATA AATCATCAAT GTTTCTAAGG 1680
CATTTATAAT AGATTATATT AATGGTTCAA AAAAAAAAAA AAAAAAAAAA AAA 1733

【図面の簡単な説明】

【図１】スギ花粉アレルゲンCry j IIのN 末端から10残基のアミノ酸配列(A)。スギ花粉アレルゲンCry j IIのN 末端から10残基のアミノ酸配列から予想されるDNA 配列(B)。スギ花粉アレルゲンCry j IIをコードするcDNAをスクリーニングするためのプローブのDNA 配列(C)。

【図２】T7 Cry j II の抗原性を、２名のスギ花粉症患者の血清を用いて、ウェスタンブロット法により同定した結果を示す。レーン１はpMGEMEX-1 （陰性対照）を保持するBL21(DE3)、レーン２はT7 Cry j Iを発現したBL21(DE3)、レーン３はT7Cry j II を発現したBL21(DE3)、レーン４はスギ花粉より精製したCry j I をそれぞれ示す。A 、B は血清の由来する患者が異なるのみで、他は同じである。

【図３】Cry j IIの全アミノ酸配列をカバーするオーバーラップペプチドを示す。

【図４】Cry j IIの全アミノ酸配列をカバーするオーバーラップペプチドを示す。

【図５】Cry j IIの全アミノ酸配列をカバーするオーバーラップペプチドを示す。

【図６】Cry j IIの全アミノ酸配列をカバーするオーバーラップペプチドを示す。

【図７】Cry j IIの少なくとも一つのT 細胞エピトープを含むペプチドを示す。

【図８】Cry j IIの少なくとも一つのT 細胞エピトープを含むペプチドを示す。

【図９】18名のスギ花粉症患者から樹立されたCry j II アレルゲンに特異的なT 細胞ラインがそれぞれ認識するオーバーラップペプチドの重要度指数を示す。

【図６】

86. (426-440) C S P C K A K L V I V H R I M
87. (431-445) A K L V I V H R I M P Q E Y Y
88. (436-450) V H R I M P Q E Y Y P Q R W I
89. (441-455) P Q E Y Y P Q R W I C S C H G
90. (446-460) P Q R W I C S C H G K I Y H P

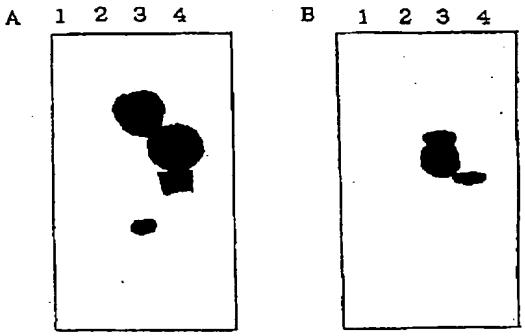
【図１】

(A) アミノ酸配列　Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr

(B) 予想されるcDNA配列　5' GCT ATT AAT ATT TTT AAT GTT GAA AAA TAT
5' GCC ATC AAC ATC TTC AAC GTC GAG AAG TAC
5' GCA ATA AAT ATA TTT AAT GTA GAA AAA TAT
5' GCG ATT AAT ATT TTT AAT GTG GAA AAA TAT

(C) 合成したプローブ　3' CGG TAG TTG TAG AAG TTG CAG CTT TTT ATG
3' CGT TAT TTG TAT AAG TTG CAC CTT TTT ATG

【図２】



１．pGEMEX-1を保持するBL-21
２．T7-CryIIaを発現したBL-21
３．T7-CryIIIを発現したBL-21
４．スギ花粉より精製したCryI

【図３】

1. (1-15) A I N I F N V E K Y G A V G D
2. (6-20) N V E K Y G A V G D G K H D C
3. (11-25) G A V G D G K H D C T E A F S
4. (16-30) G K H D C T E A F S T A W Q A
5. (21-35) T E A F S T A W Q A A C K N P
6. (26-40) T A W Q A A C K N P S A M L L
7. (31-45) A C K N P S A M L L V P G S K
8. (36-50) S A M L L V P G S K K F V V N
9. (41-55) V P G S K K F V V N N L F F N
10. (46-60) K F V V N N L F F N G P C Q P
11. (51-65) N L F F N G P C Q P H F T F K
12. (56-70) G P C Q P H F T F K V D G I I
13. (61-75) H F T F K V D G I I A A Y Q N
14. (66-80) V D G I I A A Y Q N P A S W K
15. (71-85) A A Y Q N P A S W K N N R I W
16. (76-90) P A S W K N N R I W L Q F A K
17. (81-95) N N R I W L Q F A K L T G F T
18. (86-100) L Q F A K L T G F T L M G K G
19. (91-105) L T G F T L M G K G V I D G Q
20. (96-110) L M G K G V I D G Q G K Q N W
21. (101-115) V I D G Q G K Q N W A G Q C K
22. (106-120) G K Q N W A G Q C K W V N G R
23. (111-125) A G Q C K W V N G R E I C N D
24. (116-130) W V N G R E I C N D R D R P T
25. (121-135) E I C N D R D R P T A I K F D
26. (126-140) R D R P T A I K F D F S T G L
27. (131-145) A I K F D F S T G L I I Q G L

【図４】

28. (136-150) F S T G L I I Q G L K L M N S
29. (141-155) I I Q G L K L M N S P E F H L
30. (146-160) K L M N S P E F H L V F G N C
31. (151-165) P E F H L V F G N C E G V K I
32. (156-170) V F G N C E G V K I I G I S I
33. (161-175) E G V K I I G I S I T A P R D
34. (166-180) I G I S I T A P R D S P N T D
35. (171-185) T A P R D S P N T D G I D I F
36. (176-190) S P N T D G I D I F A S K N F
37. (181-195) G I D I F A S K N F H L Q K N
38. (186-200) A S K N F H L Q K N T I G T G
39. (191-205) H L Q K N T I G T G D D C V A
40. (196-210) T I G T G D D C V A I G T G S
41. (201-215) D D C V A I G T G S S N I V I
42. (206-220) I G T G S S N I V I E D L I C
43. (211-225) S N I V I E D L I C G P G H G
44. (216-230) E D L I C G P G H G I S I G S
45. (221-235) G P G H G I S I G S L G R E N
46. (226-240) I S I G S L G R E N S R A E V
47. (231-245) L G R E N S R A E V S Y V H V
48. (236-250) S R A E V S Y V H V N G A K F
49. (241-255) S Y V H V N G A K F I D T Q N
50. (246-260) N G A K F I D T Q N G L R I K
51. (251-265) I D T Q N G L R I K T W Q G G
52. (256-270) G L R I K T W Q G G S G M A S
53. (261-275) T W Q G G S G M A S H I I Y E
54. (266-280) S G M A S H I I Y E N V E M I
55. (271-285) H I I Y E N V E M I N S E N P
56. (276-290) N V E M I N S E N P I L I N Q

【図５】

57. (281-295) N S E N P I L I N Q F Y C T S
58. (286-300) I L I N Q F Y C T S A S A C Q
59. (291-305) F Y C T S A S A C Q N Q R S A
60. (296-310) A S A C Q N Q R S A V Q I Q D
61. (301-315) N Q R S A V Q I Q D V T Y K N
62. (306-320) V Q I Q D V T Y K N I R G T S
63. (311-325) V T Y K N I R G T S A T A A A
64. (316-330) I R G T S A T A A A I Q L K C
65. (321-335) A T A A A I Q L K C S D S M P
66. (326-340) I Q L K C S D S M P C K D I K
67. (331-345) S D S M P C K D I K L S D I S
68. (336-350) C K D I K L S D I S L K L T S
69. (341-355) L S D I S L K L T S G K I A S
70. (346-360) L K L T S G K I A S C L N D N
71. (351-365) G K I A S C L N D N A N G Y F
72. (356-370) C L N D N A N G Y F S G H V I
73. (361-375) A N G Y F S G H V I P A C K N
74. (366-380) S G H V I P A C K N L S P S A
75. (371-385) P A C K N L S P S A K R K E S
76. (376-390) L S P S A K R K E S K S H K H
77. (381-395) K R K E S K S H K H P K T V M
78. (386-400) K S H K H P K T V M V E N M R
79. (391-405) P K T V M V E N M R A Y D K G
80. (396-410) V E N M R A Y D K G N R T R I
81. (401-415) A Y D K G N R T R I L L G S R
82. (406-420) N R T R I L L G S R P P N C T
83. (411-425) L L G S R P P N C T N K C H G
84. (416-430) P P N C T N K C H G C S P C K
85. (421-435) N K C H G C S P C K A K L V I

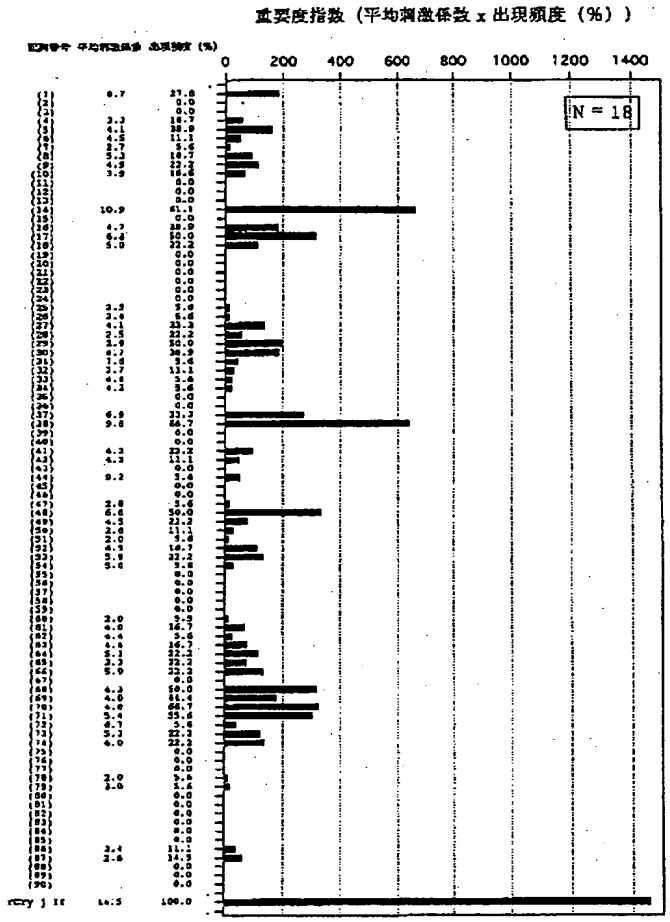
【図7】

1. Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr Gly Ala Val Gly Asp
4. Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala
5. Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala Ala Cys Lys Asn Pro
6. Thr Ala Trp Gln Ala Ala Cys Lys Asn Pro Ser Ala Met Leu Leu
7. Ala Cys Lys Asn Pro Ser Ala Met Leu Leu Val Pro Gly Ser Lys
8. Ser Ala Met Leu Leu Val Pro Gly Ser Lys Lys Phe Val Val Asn
9. Val Pro Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn
10. Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys Gln Pro
14. Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys
16. Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys
17. Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr
18. Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly Lys Gly
26. Arg Asp Arg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu
27. Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu
28. Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser
29. Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His Leu
30. Lys Leu Met Asn Ser Pro Glu Phe His Leu Val Phe Gly Asn Cys
31. Pro Glu Phe His Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile
32. Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile
37. Gly Ile Asp Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn
38. Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly
41. Asp Asp Cys Val Ala Ile Gly Thr Gly Ser Ser Asn Ile Val Ile
42. Ile Gly Thr Gly Ser Ser Asn Ile Val Ile Glu Asp Leu Ile Cys
44. Glu Asp Leu Ile Cys Gly Pro Gly His Gly Ile Ser Ile Gly Ser
47. Leu Gly Arg Glu Asn Ser Arg Ala Glu Val Ser Tyr Val His Val
48. Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe
49. Ser Tyr Val His Val Asn Gly Ala Lys Phe Ile Asp Thr Gln Asn
50. Asn Gly Ala Lys Phe Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys

【図8】

51. Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly
52. Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser Gly Met Ala Ser
53. Thr Trp Gln Gly Gly Ser Gly Met Ala Ser His Ile Ile Tyr Glu
54. Ser Gly Met Ala Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile
60. Ala Ser Ala Cys Gln Asn Gln Arg Ser Ala Val Gln Ile Gln Asp
61. Asn Gln Arg Ser Ala Val Gln Ile Gln Asp Val Thr Tyr Lys Asn
62. Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser
63. Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala
64. Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys
65. Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser Asp Ser Met Pro
66. Ile Gln Leu Lys Cys Ser Asp Ser Met Pro Cys Lys Asp Ile Lys
68. Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser
69. Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser
70. Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys leu Asn Asp Asn
71. Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe
72. Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gly His Val Ile
73. Ala Asn Gly Tyr Phe Ser Gly His Val Ile Pro Ala Cys Lys Asn
74. Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro Ser Ala
78. Lys Ser His Lys His Pro Lys Thr Val Met Val Glu Asn Met Arg
79. Pro Lys Thr Val Met Val Glu Asn Met Arg Ala Tyr Asp Lys Gly
86. Cys Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile Met
87. Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gln Glu Tyr Tyr

【図９】



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(71)Applicant : MEIJI MILK PROD CO LTD
(72)Inventor : SONE TOSHIO
KOMIYAMA NAOKI
KII KOUSUKE

(30)Priority

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05276773	05.11.1993	JP
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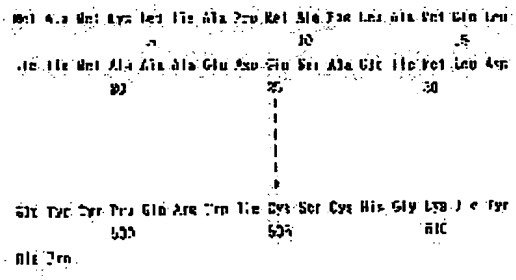
(54) ALLERGEN CRY J II EPITOPE OF POLLEN OF JAPANESE CEDAR

(57)Abstract:
PURPOSE: To obtain a new protein or peptide useful for diagnosing, preventing and treating pollinosis of Japanese cedar, containing a specific amino acid sequence and having at least one epitope of allergen of pollen of Japanese cedar, especially T cell epitope.
CONSTITUTION: This protein or peptide contains the whole or part of an amino acid sequence including an amino acid sequence of the formula, has at least one epitope of allergen Cry-j-II of Japanese cedar, especially T cell epitope and is useful for diagnosing, preventing and treating pollinosis of Japanese cedar. The protein or peptide is obtained by passing whole RNA extracted from pollen of Japanese cedar by a conventional procedure through an oligo dT cellulose column to isolate mRNA, preparing a cDNA library using the mRNA, screening the library to select a cDNA coding for Cry-j-II, then incorporating the cDNA into a manif station vector, transforming a host cell such as Escherichia coli and manifesting its gene.



LEGAL STATUS

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[Date of requesting appeal against examiner's decision of rejection]	
[Date of extinction of right]	

CLAIMS

[Claim(s)]
[Claim 1] The protein or the peptide which holds at least one epitope including all or a part of amino acid sequences of array table 1 publication.
[Claim 2] DNA which carries out the code of protein or a peptide according to claim 1.
[Claim 3] DNA according to claim 2 characterized by including all or a part of base sequences of array table 3 publication.
[Claim 4] The protein or the peptide which holds at least one epitope including all or a part of amino acid sequences of array table 2 publication.
[Claim 5] DNA which carries out the code of protein or a peptide according to claim 4.
[Claim 6] DNA according to claim 5 characterized by including all or a part of base sequences of array table 4 publication.
[Claim 7] T The protein according to claim 1 or 4 or the peptide characterized by being a cell epitope.
[Claim 8] The protein according to claim 7 or the peptide characterized by including all or a part of each following amino acid sequence.
1. Ala Ile Asn Ile Phe Asn-Val-Glu-Lys-Tyr Gly-Ala-Val-Gly-Asp4. Gly-Lys-His-Asp-Cys Thr-Glu-Ala-Phe-Ser Thr-Ala-Trp-Gln-Ala5. Thr-Glu-Ala-Phe-Ser Thr-Ala-Trp-Gln-Ala Ala-Cys-Lys-Asn-Pro6. Thr-Ala-Trp-Gln-Ala Ala-Cys-Lys-Asn-Pro Ser Ala Met Leu Leu7. Ala Cys Lys Asn Pro Ser Ala Met Leu Leu Val Pro Gly Ser Lys8. Ser Ala Met Leu Leu Val Pro Gly Ser Lys Lys Phe Val Val Asn9. Val Pro Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn10. Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys Gln Pro14.Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys16.Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys17.Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr18.Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly Lys Gly25.GluIle Cys Asn Asp Arg Asp Arg Pro Thr Ala Ile lys Phe Asp26.ArgAsp Arg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu27.AlaIle Lys Phe Asp Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu28.Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu Lys Leu M t Asn Ser29.Ile IleGln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His Leu30.Lys LeuMet Asn Ser Pro Glu Phe His Leu Val Phe Gly Asn Cys31.Pro GluPhe His Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile32.Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile33.Glu Gly ValLys Ile Ile Gly Ile Ser Ile Thr Ala Pro Arg Asp34.Ile Gly IleSer Ile Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp37.Gly Ile AspIle Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn38.Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly41.Asp Asp Cys ValAla Ile Gly Thr Gly Ser Ser Asn Ile Val Ile42. Ile Gly Thr GlySer Ser Asn Ile Val Ile Glu Asp Leu Ile Cys44.Glu Asp Leu IleCys Gly Pro Gly His Gly Il Ser Ile Gly Ser47.Leu Gly Arg Glu Asn Ser Arg Ala Glu Val Ser Tyr Val His Val48.Ser Arg Ala Glu ValSer Tyr Val His Val Asn Gly Ala Lys Phe49.Ser Tyr Val His Val AsnGly Ala Lys Phe Ile Asp Thr Gln Asn50.Asn Gly Ala Lys Phe Il AspThr Gln Asn Gly Leu Arg Ile Lys51. Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly52. Gly Leu Arg Ile Lys Thr Trp GlnGly Gly Ser Gly Met Ala Ser53.Thr Trp Gln Gly Gly S r Gly Met AlaSer His Ile Ile Tyr Glu54.Ser Gly Met Ala Ser His Ile Ile Tyr GluAsn Val Glu Met Ile60.Ala S r Ala Cys Gln Asn Gln

Arg Ser Ala Val Gln Ile Gln Asp61.Asn Gln Arg Ser Ala Val Gln Ile Gln Asp ValThr Tyr Lys Asn62.Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile ArgGly Thr Ser63. Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser Ala Thr AlaAla Ala64. Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Il Gln Leu Lys Cys65. Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser Asp Ser MetPro66. Ile Gln Leu Lys Cys Ser Asp Ser Met Pro Cys Lys Asp Ile Lys68.Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser L u Lys Leu Thr Ser69.Leu Ser Asp Ile Ser Leu Lys L u Thr Ser Gly Lys Ile Ala Ser70.Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn71.Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe72.CysLeu Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gly His Val Ile73.AlaAsn Gly Tyr Phe Ser Gly His Val Ile Pro Ala Cys Lys Asn74.Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro Ser Ala78.LysSer His Lys His Pro Lys Thr Val Met Val Glu Asn Met Arg79.Pro LysThr Val Met Val Glu Asn Met Arg Ala Tyr Asp Lys Gly86.Cys SerPro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile Met87.Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gln Glu Tyr Tyr. [Claim 9] The protein according to claim 7 or the peptide characterized by including all or a part of each following amino acid sequence.
14. Val Asp Gly Ile Ile-Ala-Ala-Tyr-Gln Asn-Pro-Ala-Ser-Trp Lys17. Asn Asn Arg Ile Trp-Leu-Gln-Phe-Ala Lys-Leu-Thr-Gly-Phe Thr29. Ile Ile Gln Gly Leu-Lys-Leu-Met-Asn Ser-Pro-Glu-Phe-His Leu38. Ala Ser Lys Asn Phe-His-Leu-Gln-Lys Asn Thr Ile Gly Thr Gly48.Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe68.Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser70.Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn71.Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe. [Claim 10] DNA which carries out the code of protein or a peptide according to claim 8 or 9.
[Claim 11] Protein or a peptide according to claim 8 or 9, the protein which has cross-reactivity immunologically, or a peptide.
[Claim 12] DNA which carries out the code of protein or a peptide according to claim 11.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] this invention is the epitope of Japan cedar pollen-allergen Cry j II useful for a diagnosis, prevention, or treatment of Japan cedar pollinosis, especially T. It is related with DNA which carries out the code of the protein containing a cell epitope, a peptide, this protein, or the peptide.
[0002]
[Description of the Prior Art] Japan cedar pollinosis is an allergic disease observed almost nationally at the beginning of spring Japan cedar pollen disperses, and presents the shape of an allergy accompanied by a sneeze, the itching of the pituita and an eye, etc. The number of patients is increasing rapidly in 1970 and afterwards, and is 10% of national [present] people. About 10 million people who hit weakness are troubled by Japan cedar pollinosis.
[0003] The allergic reaction which forms an allergic disease is R.G.H.Gell. R.R.A.Coombs I type - IV type four It is classified into the seed and Japan cedar pollinosis belongs to I type. The onset mechanism of I type allergy is as follows.
[0004] In the case of pollen, this allergen is a protein antigen although the molecule which triggers an allergic reaction is called allergen (it is also called an antigen on these specifications). If these visitor protein antigens trespass upon the inside of the body, it is incorporated by the antigen presenting cell (macrophage), and it will be decomposed by proteolytic enzyme, will become a peptide fragment, and will be shown on a cell membrane in the state where it combined with the major-histocompatibility-antigen complex (Major Histocompatibility Complex: major histocompatibility complex) class II molecule (Homo sapiens HLA class II molecule). HLA although a class II molecule shows polymorphism -- CD4+ the receptor of a T cell -- HLA the antigen peptide combined with the class II molecule -- the HLA the portion which shows the polymorphism of a class II molecule -- recognizing -- an antigen -- it is activated specifically Activated CD4+ A T cell is Th0. A cell, and Th1 / Th2 It specializes in a cell and various cytokines are produced. that time -- the cytokine production pattern of each cell -- differing -- **** -- Th1 IL-2 and IFN gamma -- Th2 IL-4, IL-5, and IL-10 etc. -- Th0 Both cytokine is produced.
[0005] On the other hand, it is B. A cell is macroglobulin to cell surface. Or immunoglobulin D It is expressing and is activated by incorporating an antigen in a cell. that time -- Th2 from -- B activated by the operation of the cytokine produced a cell -- an antibody forming cell -- specialization proliferation -- carrying out -- an antigen -- specific immunoglobulin E (immunoglobulin E) is produced Thus, produced immunoglobulin E It is immunoglobulin E to the basophilic leucocyte in a respiratory tract or the mast (obesity) cell under tunica-mucosa-nasi organization, or blood. It joins together firmly through a receptor and sensitization will be mat rialized.
[0006] Again, it is 1 when allergen tr spasses upon the inside of the body. The all rgen of a molecule is 2 on a mast cell or a basophilic leucocyt immediately. immunoglobulin E more than

a molecule It joins together and the structure of cross linkage is formed. Consequently, immunoglobulin E The receptors combined with the molecule meet, this s rves as a trigger, how many kind thing enzyme in a cell membrane is activated, and various chemical transmitters, such as a histamine, a prostagladin, and a leukotrien , are emitted from a cell. These chemical transmitters act on parts, such as tunica mucosa nasi and a respiratory tract, and cause the shape of a various allergy.

[0007] In addition, T It is T about the epitope recognized by the cell. A cell epitope and B It is B about the epitope recognized by the cell. It is called a cell epitope.

[0008] Since it is thought that the epitope of allergen is participating in an onset and exacerbation of I type allergy directly, it is useful for a diagnosis, prevention, and treatment of I type allergy to identify the epitope of allergen.

[0009] Isolation refining was done by **** and others and the main allergen of Japan cedar pollen was named Sugi Basic Protein (SBP) (Yasueda, H., et al., J.Allergy Clin.Immunol.71, 77-86, 1983). This SBP Molecular weight is 45-50kDa. WHO A nomenclature is followed and it is Present Cry j I. It is called. Furthermore, after that and Cry j I At the process of separation refining, it is Cryj I. The molecular weight from which antigenicity differs is 37kDa(s). Cry j II was separated (312 Taniai, M.et al.FEBS Letters 239, 329- 332, 1988, Sakaguchi, M.et al.Allergy 45,309- 1990).

[0010] It is Cry j I these results. Cry j II is Cry j I at a Japan cedar pollinosis patient, although it became clear that it is completely different protein. It was reported that both of Cry j II have reacted. Namely, 145 The inside of the Japan cedar pollinosis patient blood serum of a name, and 134 The blood serum of a name (92.4%) is Cry j I. And it reacts with Cry j II. 6 The blood serum of a name (4.1%) is Cry j I. It reacts and is 5. It became clear that the blood serum of a name (3.4%) reacts only with Cry j II (1993 43rd Japanese Society of Allergology per year, Hashimoto et al., the Japan Velerinary Medical Association size, ****, a national Sagamihara hospital, a wood primeval-izing **). That is, in the onset of Japan cedar pollinosis, it is Cry j I. And it was shown that Cry j II is both important.

[0011] Cry j I Cloning of the cDNA which carries out the code of it if it attaches is carried out, and it is based on the presumed amino acid sequence, and is T. The peptide containing a cell epitope is identified (WO 94/01560, "ALLERGENIC PROTEINS AND PEPTIDES FROM JAPANESE CEDAR POLLEN"). Cry j II -- N Ala of the amino acid sequence of an end, Ile, Asn, Ile, Phe, Asn, Val, Glu, and Lys And Tyr 10 amino acid residues are reported (Sakaguchi, M., et al., Allergy 45, 309-312, 1990) -- it is .

[0012]

[Problem(s) to be Solved by the Invention] this invention is at least one epitope, especially T of Japan cedar pollen-allergen Cry j II useful for a diagnosis, prevention, and treatment of Japan cedar pollinosis. It aims at offering DNA which carries out the code of the protein containing a cell epitope, a peptide, this protein, or the peptide.

[0013]

[Means for Solving the Problem] Invention-in-this-application persons are (1) Cry j II, in order to solve the above-mentioned technical problem. Elucidation Cry j II of all amino acid sequenc s (primary structure) (2) Production Cry j II of the overlap peptide which covers all amino acid sequences (3) T which recognizes allergen specifically It is establishment (4) according to an individual about a cell line. The overlap peptide containing the establishment (5) T-cell epitop of an antigen presenting cell (B cell strain) was identified, and this invention was completed. In addition, the epitope as used in the field of this invention is T. It is Following T although not restricted to a cell epitope. A cell epitop is explained in full detail. Each of these st ps are explained below.

[0014] (1) Cry j II Extraction RNA of cloning a.RNA of elucidation ** cDNA of all amino acid sequences In cas it extracts, protein is usually removed by the initial stage. For this r ason, th re is a method using prot in denaturation ag nts, such as the phenol extraction m thod, a

GUANIJIUMU salt, a surfactant, and a urea, etc. as common practice.
[0015] RNA from Japan cedar pollen extraction -- Breiteneder ** (Int.Arch.Allergy Appl.Immunol.87:19-24 and 1988) -- improvement can be added and can be carri d out to a method
[0016] Japan cedar pollen is suspended ten to 20 times in the extraction buffer solution (100mM LiCl, 10mMNa2 EDTA, 1%SDS, 20% mercaptoethanol, and 100 mM Tris-HCl pH 9.0) of an amount, and ize [of an equivalent phenol and the mixture (phenol : chloroform : isoamyl alcohol = 24:24:1) of chloroform / HOMOJIE] is added and carried out to this. Subsequently, centrifugal (10,000g, 10 - 15 minutes) is carried out, and it separates into a phenol chloroform layer and the bilayer of a water layer. The protein which denaturalized at this time shifts to a phenol chloroform layer, and a nucleic acid shifts to a water layer. Impurities, such as protein which adds and shakes a phenol chloroform mixture to a water layer, and remains in the water layer, are made to shift to a phenol chloroform layer, and are removed. Such operation is repeated twice.
[0017] the obtained water layer to RNA for extracting -- high-concentration LiCl (2-4M) or -- if CH3 COONa (3M) exists -- DNA and protein -- a supernatant liquid -- remaining -- RNA other than tRNA The property to precipitate is used. LiCl of 2 -4M of the amount of said is added to a water layer, and it is RNA. It is made to precipitate. Subsequently, this water layer is dissolved in water and it is 0.1-0.3. The cold ethanol (-20 **) of ** is added, and it is RNA. It is made to precipitate (ethanol precipitation). Subsequently, centrifugal (10,000g, 30 minutes) is carried out, precipitation is collected, and it dissolves in water, and is all RNA. Fractionation is obtained.
[0018] b. mRNA mRNA of the composition Cry j II of manufacture and cDNA is poly (A) to a three-dash terminal. Since it has a chain, mRNA is adsorbed by the oligo-dT-cellulose column (Clontech Laboratories Inc. company make, CA, USA) which combined the deoxythymidine (dT) of 12 to 18 base as a ligand which carries out the complementation to this. Japan cedar pollen RNA The buffer solution (3M NaCl, 1mM EDTA, 10 mM Tris-HCl, pH 7.4) is added, and mRNA is made to stick to a column. mRNA is 2-3 of bed volume. It is eluted with the buffer solution (1mM EDTA, 10 mM Tris-HCl, pH 7.4) which does not contain NaCl of the amount of double.
[0019] Production of the cDNA library from obtained mRNA can perform the phage marketed now using the cDNA library production kit (Amersham International plc. company make, Buckinghamshare, England) used for the vector.
[0020] c. N of the screening Cry j II of Cry j II cDNA Synthetic DNA which has the base sequence presumed from this amino acid sequence although end amino acid 10 residue has already become clear It considers as a probe and is Cry j II cDNA. It can screen. DNA used for a probe When compounding, it is more desirable to design an oligonucleotide which is hybridized to two or more possible codon arrays rather than compounding all the oligonucleotides containing a possible codon. The indicator of the five prime end of this synthetic oligo NUKUREOCHIDOPU lobe is carried out to [gamma-32P] ATP by the polynucleotide kinase, and an electropositive clone is screened from the aforementioned cDNA library by the plaque hybridization method.
[0021] the obtained electropositive clone -- phage DNA preparing -- an insertion cDNA fragment -- dissociating -- pUC18 etc. -- a sub clone is carried out to a plasmid An oligonucleotide primer is compounded if needed, a base sequence is determined by the Sanger method etc., and a clone is identified. Cry j II cDNA which this invention persons isolated The base sequence of an overall l ngth is shown in the array number 5.
[0022] cDNA which carries out th code of Cry j II consists of 1733bp(s) on the whole, contains the open reading fram from the codon (nucleotide ATG of th 45-47th place) assumed to be initiation to a stop codon (nucl otide TAA of th 1587-1589th plac), and is 514. The code of the amino acid is carried out. The amino acid s qu nc in which shows the base s quence of an open-reading-frame portion to the array number 3, and this base sequence carries out a code is

shown in the array number 1. Polymorphism according to the allele variation betw en individuals in the base sequence shown by the array number 3 (polymorphism) And although th variation of the amino acid sequence as the result can be considered, the base sequence and amino acid sequence of Cry j II which has such a variation are also included by this invention. 207 -236 DNA of grade the amino acid sequence in which an array carries out a code -- Ala, Ile, Asn, Ile, Phe, Asn, Val, Glu, Lys, and Tyr it is -- N of matured type Cry j II It is in agreement with an end amino acid sequence (Sakaguchi, M., et al., Allergy 45, 309-312, 1990). N Since 54 amino acid of an end is rich in the hydrophobic amino acid looked at by other transit peptides and is not contained in matured type Cryj II, it is considered to be a transit peptide.

[0023] 207 DNA from grade to the 1587-1589th place of a stop codon Cry j II in which an array carries out a code -- N Ala of an end from -- C Pro of an end up to -- 460 It consists of an amino acid residue of an individual, and is considered matured type Cryj II. The amino acid sequence by which a code is carried out to the array number 4 at this base sequence in the base sequence corresponding to this matured type Cry j II is shown in the array number 2. The molecular weight on the theory of Cry j II which consists of an amino acid sequence shown in the array number 2 is 50,444Da(s). On the other hand, natural matured type Cry j II is 45KDa(s) at the SDS-polyacrylamide electrophoresis under reduction conditions (SDS-polyacrylamide gel electrophoresis). The band appears in a position (Sakaguchi, M., et al., Allergy 45, 309-312, 1990). C of this to Cry j II It is thought that the end has received processing. Moreover, Asn-X-Ser/Thr which has the possibility of N-glycosidic linkage in the amino acid sequence of Maturation Cry j II It exists.

[0024] DNA which carries out the code of Cry j II DNA containing an overall length or its part DNA which carries out the code of the protein which includes biochemical inspection, related protein, or an analogous array by carrying out an indicator by the fluorescence indicator, the radioactive indicator, or enzyme labeling It can be used as the probe for screening etc., and a primer. Moreover, it can connect with an expression vector and the protein or the peptide containing at least one epitope can also be made to discover.

[0025] ** Manifestation rCry j II of recombination Cry j II (rCry j II) Or the recombination protein or the peptide containing the epitope of at least one Cry j II includes cDNA which carries out the code of each in an expression vector, and it can be introduced into Escherichia coli, an insect cell, yeast, or mammalian, and it can obtain it by cultivating. However, the manifestation system using prokaryotic cells, such as Escherichia coli, is addition (glycosylation) of a suitable sugar chain. Since it is not carried out, it is rCry j II. It may be desirable to use eukaryotic cells, such as yeast, for a manifestation.

[0026] The example of some manifestation systems of Cry j II is shown below.

[0027] a. The promotor and RNA of manifestation T7 phage in Escherichia coli A polymerase the system (it Studier(s) F.W. --) to be used A. H.Rosenberg and J.J. Dunn and J.W.Dubendonff, "Methods in Enzymology", ed.by D. D.V.Goeddel, vol.185, p.60, Academic Press, New York, and 1990 Since the success percentage of a manifestation is very high, it can be used suitable for this invention. This system is the Escherichia coli host BL21 (DE3) with the polymerase gene of T7 phage. It is the system which inserted the target gene in the multi-cloning site of T7 phage promotor's lower stream of a river and which it rearranges [system], and a plasmid is introduced [system] and makes the target gene discover under IPTG existence. For example, pGEMEX-1 (Promega) etc. can be used as an expression vector.

[0028] Moreover, the system which unites the target protein with the protein which can be discovered extensive, and mak s it discover is marketed, and these systems can use an affinity column for refining, and its refining efficiency is good and can use them suitable for this invention. For example, it is beta to a fusion protein. - If expression vector pUEX (Amersham) which has galactosidas is used, it is rCry j II. b ta - It is obtained as a fusion protein with galactosidase, and can refine efficiently by the affinity column. Moreover, th cleavage site of a blood coagulation factor Xa is introduc d into the fusion zone grade, and pGEX (Pharmacia)

which has a glutathione S-transferase, pMAL (New England Biolabs, Berverly, MA) using the maltose binding protein, etc. can separate Cry j II.
[0029] b. The glycosylation of a manifestation product is possible for the system which makes manifestation yeast in yeast a host, and this is convenient to the manifestation of Cry j II which is glycoprotein. For example, as a manifestation system of the foreign protein by yeast, the method of using the Pichia yeast as a host is learned, and it can be used suitable for this invention (JP,61-108383,A, JP,61-173781,A, JP,63-44899,A, JP,1-128790,A, etc.). About the manifestation system by other yeast, they are D.Emr Scott, "Methods in Enzymology", ed.by D.V.Goeddel, vol.185, p.231, Academic Press, and New York (1990). It is explained in full detail and can be used by this invention.
[0030] c. The glycosylation of a manifestation product is possible for the system which makes a host the inside of the manifestation insect cell in an insect cell. The foreign gene manifestation system using the baculovirus is marketed (PharMingen, San Diego, CA, USA), and can be used suitable for this invention. this system -- Luckow and V.A. ** -- It is indicated by Trends in the Development of Baculovirus Expression Vector and Bio/Technology (1987 year 9 moon 11 day).

[0031] d. It can include in an expression vector with the manifestation mammals promotor (for example, metallothionein) in a mammalian cell, a virus promotor (for example, initial promotor of simian virus 40), etc., and can be made high-discovered by introducing into a mammalian cell.
[0032] (2) T of the synthetic pollinosis patient of an overlap peptide T of Cry j II which a cell recognizes Cry j II cDNA given in the array number 2 in order to solve a cell epitope on molecule level the amino acid sequence which carries out a code -- being based -- N Ala of an end from -- C Pro of an end it results -- all -- 460 The overlap peptide which covers an amino acid residue is produced. These overlap peptides are easily compoundable with the peptide automatic synthesizer unit marketed. The peptide containing at least one epitope is identified out of these overlap peptides. Especially, it is T. T which recognizes Cry j II specifically and carries out a proliferation response from a pollinosis patient's peripheral blood lymphocyte in order to identify a cell epitope It is necessary to establish a cell line. T which generally reacts for every patient Since cell epitopes differ, it is T for every patient. It is desirable to establish a cell line.
[0033] (3) the establishment Cry j II antigen of a T cell line -- specific T for establishing a cell line -- usually -- a patient's peripheral blood lymphocyte -- the bottom of existence of a Cry j II antigen, and 7 between [days] grade cultivation -- carrying out -- an antigen stimulus -- T a cell -- being activated -- further -- activation T a cell -- an antigen and an antigen presenting cell -- 7 repeat cultivating during a day several times and it carries out an antigen stimulus -- antigen specific T A cell line is producible. However, T When the cell is increasing well under existence of IL-2 of a growth factor, as for an antigen stimulus, carrying out only first is desirable. T If the abundance antigen stimulus of the cell line is carried out, it is high T of a growth ratio. A cell can be taken alternatively and it is T. The case where sufficient proliferation response is not shown depending on an epitope when identifying the peptide containing a cell epitope arises.
[0034] As an antigen to be used, although a nature type Cry j II antigen is theoretically desirable, since only a ultralow volume can be extracted from Japan cedar pollen, the mixture of recombination Cry j II (rCry j II) or an overlap peptide can also be used suitably. rCry j II What was made to discover by Escherichia coli and was refined can be used.
[0035] (4) As an establishment antigen presenting c ll of an antigen presenting cell (B cell strain), it is T. About th p ripheral blood lymphocyte of the same people as a cell line, it is mitomycin C. Processing or the thing which radiation irradiation was carri d out [thing] and made the proliferation pot ntial force lose is desirable. How v r, since the number of times of blood collecting increases, it is s lf B about Epstein-Barr virus (EBV). Since it continues increasing by in vitro and becomes a lymphoblast Mr. cell strain (B c ll strain), the thing which

made it infected with a lymphocyte and made transformation start is this B. You may use a cell strain as an antigen presenting cell. B The establishment method of a cell strain is already established [the volume the technical second edition of tissue culture, 187–191 page, and on Japanese histology meeting (1988. 8.10)].

[0036] (5) T peculiar to the patient of each identification of the overlap peptide containing a T cell epitope T which a cell line recognizes The peptide containing a cell epitope is identified as follows. The meaning of "recognizing" here is T. A cellular receptor combines with an antigen epitope (including a major–histocompatibility–complex molecule) specifically, consequently it is T. Meaning that a cell is activated, the state of activation is [production of lymphokine, and] DNA. Composition [3H] It is observed by measuring the amount of incorporation of thymidine as an index. Namely, T A cell line and mitomycin C B of the same people who processed Seeding of the cell strain is carried out to 96 hole flat bottom plate, and it carries out mixed culture with an overlap peptide. [3H] The amount (cpm) of incorporation of thymidine is measured with a liquid scintillation counter. In that case [3H] The incorporation of thymidine T to each peptide since it differs by each cultivation system Cell line [3H] The amount (cpm) of thymidine incorporation Control which has not added the antigen [3H] The number (stimulation index: SI) which **(ed) in the amount (cpm) of thymidine incorporation is 2. It is T about the above. The peptide containing a cell epitope is identified. Identified T The peptides containing a cell epitope are enumerated by drawing 4 .

[0037] Thus, at least one T of Cry j II of the obtained this invention The following things can b considered about the peptide containing a cell epitope. HLA Since it is thought from the analysis result (Chicz, R.M.etal.:J.Exp.Med., 178:27–47, and 1993) of a peptide that it consists of about ten to 34 amino acid residue, the length of the peptide by which combines with a class II molecule and antigen presentation is carried out is T of this invention. As for the peptide containing a cell epitope, the peptide of such length is also contained. Moreover, T for every patient [as opposed to / embellish an amino acid substitution, a deletion, or addition to the peptide of this invention, and / these ornamentation peptides] By measuring the proliferation response of a cell line, it is at least one T of Cry j II of this invention. Since producing easily the peptide containing a cell epitope and the ornamentation peptide which has this function immunologically is that this contractor can carry out easily, these ornamentation peptides are also included by this invention.

[0038] Now, the hyposensitization agent currently used by hyposensitization is the rough antigen extracted from Japan cedar pollen, and contains a lot of polysaccharide. Once there is a lot difference considerably and it starts hyposensitization, when a lot is changed, anaphylaxis is rarely started. Moreover, it is about 30% that it has seldom been improved but the curative effect of hyposensitization has also been diagnosed as higher efficacy by hyposensitization since hyposensitization treatment was started. It is a patient.

[0039] T of this invention T more than the half of the pollinosis patient among the peptides containing the epitope of a cell As for a cell line and each peptide which reacts, hyposensitization may be able to perform each of these peptides independent or above the half of the patient who treated when hyposensitization was performed using the peptide which mix d some. Moreover, since the peptide to be used is a peptide compounded chemically, it is thought that possibility of producing a side effect like anaphylaxis becomes low. For example, drawing 5 is T established from 18 pollinosis patients. The overlap peptide which a cell line recognizes, respectively "frequency of occurrence (%) with significance index ["an average stimulus coefficient" ("— overlap — a peptide — a stimulus — d pending — T — a cell — a line — — [— three — H —] — thymidine — incorporation — an amount (cpm) — " — "— an antig n — not adding — a case — — [— three — H —] — thymidine — incorporation — an amount (cpm) — " — having divid d — a value — the average) Although value] which multiplied by " ("T c II line which has recognized th subj ct peptide as a T c II epitope" to "all examined T cell lines" is (%) comparatively) shows th peptid of the numbers 14, 17, 29, 38, 48, 68, 70, and

71 in drawing -- an average stimulus coefficient -- about 3.9 a significance ind x when it is above -- 200 It has exceeded and it is thought that it is especially effective in hyposensitization treatment.
[0040] In addition, at least one T with which this invention person clarified and was indicated to be to drawing 5 B of the after-mentioned [inside / of the peptide containing a cell epitope] Two kinds of peptides which that a cell epitope is included made clear, and the peptide which has an intersection are not contained. Therefore, the peptide of this invention is B. Since it is thought that a cell epitope is not stimulated, it thinks [that it is utilizable as a hyposensitization agent, and].
[0041] Moreover, T of this invention Internal use of the peptide containing the epitope of a cell is carried out, and it is thought possible to perform peroral-immunity tolerance. Although peroral-immunity tolerance (oral hyposensitization) is a cure under development now, the result which takes effect is beginning to be reported. For example, T of Myelin Basic Protein If internal use of the cell epitope (the peptide array 21-40 and 71-90) is carried out to a mouse, having suppressed "the Experimental Autoimmune Encephalomyelitis onset (omitting EAE)" is reported [the molecular biology of Shuichi Uenokawa, the Hisatsune ****, Toshiyuki Yamura, and peroral-immunity tolerance, a protein nucleic-acid enzyme, 39, and 2090-2101 1994 (written page 2098 right, nine to 24 lines)]. T identified also in Japan cedar pollinosis from these examples If it devises enclosing with a certain capsule etc. and internal use is carried out so that internal use of the cell epitope peptide may be carried out as it is or it may not be digested with the stomach, it may be in an immunological-tolerance state. It is specifically 12-1 before a Japan cedar pollen scattering stage. An epitope peptide is prescribed for the patient in taking orally, and the immunological-tolerance state is guided at the moon term. If it is in this state, even if Japan cedar pollen will disperse and pollen will adhere to the tunica mucosa nasi, a symptom does not come out, or a symptom is light and a bird clapper is expected.
[0042] It is T of this invention further again. The analog peptide which added ornamentation of an amino acid substitution, a deletion, or addition to the peptide containing the epitope of a cell is compounded, and it is HLA. It is T although it combines with a class II molecule. The analog peptide from which information is not transmitted is identified into a cell. These peptides will be natural T if it is used for a patient as collunarium. Since a cell epitope is checked in competition, onset prevention is expected.
[0043] In addition, a B cell epitope is also contained in the epitope as used in the field of this invention. Identification of a B cell epitope is an overlap peptide and the patient blood serum immunoglobulin E. It can carry out by well-known methods, such as reactant measurement with an antibody, and detection of prevention of combination with the patient blood serum and antigen by the overlap peptide, (refer to JP,6-69336,A). Univalent B already It is known that a c ll epitope is useful to suppression of an allergic reaction. This is univalent B. A cell epitope is immunoglobulin E to which it corresponds on a mast cell or a basophilic leucocyte. immunoglobulin E combine with a molecule and according to a multiple-valued epitope It is thought that it is because formation of molecule bridge formation is checked. This invention persons compound the overlap peptide which covers all the amino acid sequences of Cry j II of this invention. These peptides and Japan cedar pollinosis patient blood serum immunoglobulin E The result which measured the reaction with an antibody by enzyme antibody technique, "A peptide Gln Cys Lys Trp Val Asn Gly Arg Glu Ile Cys (amino acid sequence 113-123)" and "Cys Thr Ser Ala Ser Ala Cys Gln Asn" (amino acid sequence 293-301) -- B A cell epitope It was shown clearly that it contains. B of such Cry j II The peptide containing a cell epitope is useful for a diagnosis, prevention, and treatment of Japan cedar pollinosis.
[0044]
[Example] Although this inv ntion is explained in detail based on an example below, this invention is not limit d to this.
[0045] <Extraction of Japan c dar pollen> Japan cedar poll n is 2 at Shizuoka Prefecture and a

Kanagawa within the prefecture. It extracted from the male which carried out flower setting to the branch of the Japan cedar cut down in the moon. Cryj II It saves by −70 **, and the Japan cedar pollen for antigenicity refining is RNA. After quick−freezing the Japan cedar pollen for manufacture in liquid nitrogen, it was saved by −70 **.

[0046] <RNA Extraction >Breiteneder ** (Int.Arch.Allergy Appl.Immunol.87:19−24 1988) adding improvement based on a method −− Japan cedar pollen to RNA It extracted.

[0047] It suspends in the 15ml extraction buffer solution (100mM LiCl, 10mMNa2EDTA, 1%SDS, a 20% 2−mercaptoethanol, 100 mM Tris−HCl, pH 9.0) which ice−cooled 1g of Japan cedar pollen which carried out cryopreservation, and is 15ml phenol:chloroform:isoamyl alcohol (24:24:1) further. It added. Ize [20−30 stroke HOMOJIE] was carried out having moved this suspension to the Teflon homogenizer and turning TEX ROMPE stere by the highest rotation by the motor. Then, centrifugal operation (10,000g, 15 minutes) It separated into the water layer and the organic layer, and the water layer was obtained. a water layer −− phenol [of the amount of said]: −− chloroform: −− isoamyl alcohol −− adding −− 5 a part −− after between shaking and centrifugal separation (10,000g, 15 minutes) The water layer was obtained. It is the same operation 2 A time repeat, 15 moreml chloroform: Use isoamyl alcohol (24:1) and it is 1. ********. They are 4M LiCl of the amount of said to the obtained water layer. It added and was left in −20 ** overnight. The frozen solution is dissolved at a room temperature and it is centrifugal operation (20,000g, 30 minutes). Sedimentation was obtained. This sedimentation is dissolved in a little sterile distilled water, and it is 0.3. 3M CH 3 COONa of **, and pH 5.2 and 2.5 The ethanol of ** was added and it was left for 60 minutes in −20 **. Centrifugal operation (10,000g, 30 minutes) The collected dregs are remelted to a sterile distilled water, and it is all RNA. It considered as fractionation.

[0048] <composition of Japan cedar pollen mRNA manufacture−and cDNA> Japan cedar pollen −− all −− RNA1mg −− start material −− carrying out −− the joint buffer solution (3 M NaCl) of the amount of said 1mM EDTA, 10 mM Tris−HCl, and pH 7.4 After adding, the span column (CLONETECH Laboratories Inc. company make −−) which packed oligo dT cellulose in advanc It is made to stick to CA and USA and is the elution buffer solution (1mM EDTA, 10 mM Tris−HCl, and pH 7.4). It is about 10microg by being eluted. mRNA was refined (the CLONETECH Lab.Inc. company appending protocol was followed). Then, refining mRNA 5microg Shell cDNA composition system plus (Amersham International plc. company make, Buckinghamshare, England) is used, the protocol appended is followed, and it is cDNA 4 [about]. mug It compounded.

[0049] N of the <composition of an oligonucleotide probe> Cry j II The amino acid sequence of ten residues is shown in drawing 1 A from an end. The array of cDNA expected from this amino acid sequence is drawing 1 B. Since two kinds of bases were used for the array by four places complementary as an oligonucleotide probe (Oligo CJII), it compounded as a total of 16 kinds of mixture (drawing 1 C). It is G:T in order to reduce a kind as mixture. The base pair is permitt d.

[0050] <Cry j II cDNA Production of a cloning >cDNA library used cDNA cloning system lambdagt10 (Amersham International plc. company make, Buckinghamshare, and England), and p rformed it according to the protocol appended. Above−mentioned cDNA 1microg It included in lambdagt10 and the cDNA library was produced. Inside 5,000 [about] of about 500,000 library It is the diameter of 150mm about a clone. It wound around one plate. The probe for screening carried out the indicator of the above−mentioned oligonucleotide (Oligo CJII) by T4 polynucleotide kinase by [gamma−32P] ATP (7,000 Ci/mmol ICN Biochemicals, Inc. company make), and used it. It is the nitrocellulose filt r which fixed phage DNA 5 xsubacute scleroting panencephaliti (1 xsubacute−scleroting−panenc phaliti:0.18M NaCl, 10mM sodium phosphate, 1mM EDTA), 5 xFBP (1 xFBP:0.02% Ficoll, 0.02% cow serum albumin, 0.02% polyvinyl pyrrolidone), 0.3%SDS, and 100 mug/ml tRNA It is 1 48 degrees C to the includ d solution. It pre hybridiz d by dipping beyond time. It dips in this solution which newly prepared the nitrocellulos filter after this, and is 32P. The probe (Oligo CJII) which carried out the label was added, and

hybridization was performed at 48 degrees C overnight. They are 6 xSSC (1 xSSC:0.15M NaCl, 0.015M sodium citrate) and 0.1%SDS about a filter after this. It is 5 48 degrees C the room temperature of 30 degrees C with the included solution. Autoradiography was performed after part-washing. 4 The strong signal of an individual is detected and it is one of phage DNA of it. It extracts and is a restriction enzyme EcoRI. When cut, it is DNA of about 1.7 Kbp(s). It became clear that the fragment is inserted. Sub cloning of the insert was carried out to pUC118, the delay SHON mutant was produced using the kilo sequence DERESHON kit (TAKARA SHUZO CO., LTD. make), and it used for the determination of all base sequences. The base sequence performed the primer extension reaction using the synthetic primer and the coloring matter indicator dideoxy terminator, and determined it by reading by the automatic sequencer (model 370A, Applied Biosystems, and Japan). All the determined cDNA base sequences are shown in the array number 5. moreover, the base sequence which carries out the code of the maturation Cry j II for the base sequence of only an open reading frame to the array number 3 (the amino acid sequence in which this base sequence carries out a code -- the array number 1) is shown in the array number 4 (the amino acid sequence in which this base sequence carries out a code -- the array number 2)

[0051] <Manifestation by the Escherichia coli of recombination Cry j II> Promega It is 1TEscherichia coli expression vector pGEMEX-7 promotor and T7 gene10 which are marketed from the shrine. It has coding sequence and T7 terminator, and is T7 gene10 about an open reading frame. It inserts in a down-stream multi-cloning site, and is T7. It is the vector which performs a high manifestation by introducing into the Escherichia coli (Example BL21 (DE3)) which discovers RNA polymerase. Cry j II cDNA It digests by BamHI (the adapter connected with the ends of cDNA includes a BamHI site), cDNA fragmentation is started, and it is BamHI of pGEMEX-1. It includes in a site and is expression vector pEXCJII of Cry j II. It built. pEXCII is T7 gene10. The fusion protein (T7 Cry j II, 73kD) of a manifestation product (23kD) and Cry j II protein (50kD) may be discovered. It is Escherichia coli BL21 (DE3) about pEXCII. The introduced transformant is cultivated and it is T7 at IPTG. RNA polymerase was guided and Cry j II was discovered. It is SDS about the cell extract of the discovered Escherichia coli. It applied to polyacrylamide gel electrophoresis. BL21 (DE3) holding pEXCII **** -- T7 Cry j II of about 73 kD(s) ** -- the band considered was seen however, BL21 (DE3) holding pGEMEX-1 of contrast Or old stock BL21 (DE3) **** -- these bands were not seen

[0052] <Cry j II and T7 gene10 Reactant >T7 Cry j II with the Japan cedar pollinosis patient blood serum of fusion protein (T7Cry j II) About the extract of the discovered Escherichia coli, it is SDS. Millipore after carrying out polyacrylamide gel electrophoresis Western blotting (West rn Blotting) is carried out to a shrine PVDF film, and it is the Japan cedar pollinosis patient 5. People and healthy people 3 Reactivity with people's blood serum was examined. BL21 and T7 gene10 which hold pGEMEX-1 as contrast Cry j I Nature type Cry j I refined from the extract of BL21 which discovered the fusion protein (T7Cry j I), and Japan cedar pollen The blot was carried out simultaneously and the reaction was investigated. It is 2 as shown in drawing 2 . People's patient blood serum is T7 Cry jII. It reacted. 2 People's patient blood serum is T7 Cry j II and a nature type. Although it has reacted to Cry j I, it has not reacted to BL21 extract and T7 Cry j I holding pGEMEX-1. These results to T7Cry j II is immunoglobulin E in a Japan cedar pollinosis patient blood serum. Having the antigenicity which reacts was checked.

[0053] Composition of a <composition of overlap peptide> exaggerated peptide was performed using Peptide Synthesizer PSSM -8 (Shimadzu make). a basis [primary structure / of Cry j II / which is shown in the array number 2] -- carrying out -- N an end side -- 55th Ala from -- starting -- C Pro of an end up to -- 90 kinds of ov rlap peptides of 15 **** containing the overlap portion of ten residues w re compounded 1 charact r code of amino acid is used for drawing 3 -6, and all the compound overlap peptides are shown.

[0054] <B the peripheral blood lymphocyte (1x106) obtain d by th establishm nt >Ficoll-Paque specific gravity centrifugation of a cell strain -- about -- Epstein-Barr virus (EBV) of 1 x106

PFU (plaque forming units) -- 37 degrees C -- 1 Time incubation was carried out and the virus was infected with the cell. This virus infection cell is moved to 24 well cultivation plate, and it is 100 ng/ml cyclosporin A. It is 2 under existence. It is B if it cultivates before or after a week. A cell colony appears. It divided into the half at this time, and it planted in the n w well and inherited. If this operation is repeated successively and subculture is performed, it is B in which self-multiplication is possible. A cell may appear. This self-multiplication B 25cm2 after carrying out IKUSUPANDO (expand) of the cell of the well containing a cell and checking proliferation It moves to a culture flask, cultivation is performed for further 30 - 50 days, and it is EBV. B which it transformed (EBV-transformed) The cell strain was obtained. B Cryopreservation of a part of cell strain was carried out.

[0055] <Cry j II antigen specific T It saved in liquid nitrogen until it isolated and used it by the Ficoll-paque specific gravity centrifugation usually used in the lymphocyte from the 18 establishment > Japan cedar pollinosis patient peripheral blood of a cell line. a Japan cedar pollinosis patient's peripheral blood lymphocyte (4 x106 individual) -- 20% of self plasma of 2 ml RPMI-1640 added suspended, and it was made discovered by 10microg [/ml] Escherichia coli, and refined -- rearranging -- a Cry j II antigen -- 24 hole cultivation plate top -- 7-8 It cultivated during the day. T activated in response to the Cry j II antigen stimulus (a ****** reaction and blastogenesis) It is 5 Unit/ml when the cell has checked under the microscope. IL-2 were added and it cultivated overnight. They are 20 Unit/ml IL-2 from the next day, and 20%. RPMI-1640 which added the Homo sapiens AB type blood serum (commercial elegance) It is 9, replacing culture medium with every day. It cultivated during the day. Increased T which recognizes a Cry j II antigen specifically at this time Cryopreservation of a part of cell line was carried out. Furthermore, it is T. It is a cell line in the above-mentioned culture medium 4 It cultivated during the day and considered as the cell for identification of an epitope.

[0056] <T T established from the identification >18 person pollinosis patient of the exaggerated peptide containing a cell epitope It cultivates with a Japan cedar pollen-allergen overlap peptide about a cell line, respectively, and is Cry j II antigen specific T. The peptide containing a cell epitope was identified.

[0057] T Cultivation B established from the same patient as a cell line It is 50microg [/ml] mitomycin C about a cell strain. It processes for 30 minutes and is a cell RPMI-1640 4 Time washing was carried out. This B After carrying out seeding (5 x104 / well) of the cell to 96 hole flat bottom plate (96-well flat-bottomed plate), it added to each well and Cry j II (the 25microg [/ml] last concentration) or each overlap peptide (last concentration 0.5 muM) was cultivated for about 60 - 90 minutes. T Carry out seeding of the cell line (2x104 / well) to each well, and it is after 48-hour cultivation and 0.5. It added to the well and mul/Ci [3 H] thymidine was cultivated for further 16 hours. The uptake of the cell was carried out on the glass filter using the cell harvester, and after drying, the count (cpm) of [3 H] thymidine incorporated in the cell was measured with the liquid scintillation counter.

[0058] Measurement is performed by triplicate culture. a result T by overlap peptide stimulus The amount (cpm) of [3 H] thymidine incorporation of a cell line It computes by the stimulus coefficient (stimulation index; SI) which is the value broken by the amount (cpm) of [3 H] thymidine incorporation when not adding an antigen (control). SI is 2. About the overlap peptide which showed the above value, it is T. The exaggerated peptide containing a cell epitope was identified. Drawing 7 and drawing 8 are Cry j II antigen specific T established from 18 pollinosis patients, respectively. At least one T which at least one kind of a cell line recognizes The peptide containing a cell epitope is shown. Moreover, drawing 9 shows th "average stimulus coefficient" (average of the stimulus coefficient obtained by two or more experim nts) and "the frequency of occurrence (%)" of all overlap peptides, and the "significance index."

[0059]

[Effect of the Inv ntion] The peptide and T containing at least one epitope of Cry j II of this invention The peptide containing a cell epitope is us ful for a diagnosis, prevention, and

treatment of Japan cedar pollinosis.
[0060] It is HLA further again. Class It is T although it combines with a mol cul . It is also possible to compound an analog peptide from which information is not transmitted to a cell, and to use these peptides for the Japan cedar pollinosis onset prevention by competitive inhibition.
[0061]
[Layout Table]
array number: -- length [of one array]: -- mold [of 514 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- protein array: -- Met Ala Met Lys Leu Ile Ala Pro Met Ala Phe Leu Ala Met Gln Leu 5 10 15 Ile Ile Met Ala Ala Ala Glu Asp Gln Ser Ala Gln Ile Met Leu Asp 20 25 30 Ser Val Val Glu Lys Tyr Leu Arg Ser Asn Arg Ser Leu Arg Lys Val 35 40 45 Glu His Ser Arg His Asp Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr 50 55 60 Gly Ala Val Gly Asp Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr 65 70 75 80 Ala Trp Gln Ala Ala Cys Lys Asn ProSer Ala Met Leu Leu Val Pro 85 90 95 Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys 100 105 110 Gln Pro His PheThr Phe Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln 115 120 125 Asn Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys 130 135 140 Leu Thr Gly Phe Thr Leu Met Gly Lys GlyVal Ile Asp Gly Gln Gly 145 150 155 160 Lys Gln Trp Trp Ala Gly GlnCys Lys Trp Val Asn Gly Arg Glu Ile 165 170 175 Cys Asn Asp Arg AspArg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr 180 185190 Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His 195 200 205 Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile 210 215 220 Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp Gly Ile Asp Ile Phe Ala 225 230 235 240 Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly Asp Asp 245 250 255 Cys Val Ala Ile Gly Thr GlySer Ser Asn Ile Val Ile Glu Asp Leu 260 265270 Ile Cys Gly Pro Gly His Gly IleSer Ile Gly Ser Leu Gly Arg Glu 275 280 285 Asn Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe 290 295 300 Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser 305 310 315 320 Gly Met Ala Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile Asn Ser 325 330 335 Glu Asn Pro Ile Leu Ile Asn Gln Phe-Tyr-Cys-Thr-Ser-Ala-Ser-Ala 340 345 350 Cys Gln Asn Gln Arg Ser Ala Val Gln-Ile-Gln-Asp-Val-Thr-Tyr-Lys 355 360365 Asn Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys 370 375 380 Ser Asp Ser Met Pro Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu 385 390 395 400 Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn 405 410 415 Gly Tyr Phe Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro 420 425 430 Ser Ala Lys Arg Lys Glu Ser Lys Ser His Lys His Pro Lys Thr Val 435 440 445 Met Val Glu Asn Met Arg Ala Tyr Asp Lys Gly Asn Arg Thr Arg Ile 450 455 460 Leu Leu Gly Ser Arg Pro Pro Asn Cys Thr Asn Lys Cys His Gly Cys 465 470 475 480 Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gln 485 490 495 Glu Tyr Tyr Pro Gln Arg Trp Ile Cys Ser Cys His Gly Lys Ile Tyr 500 505 510His Pro array number: -- length [of two arrays]: -- mold [of 460 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- protein array: -- Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr Gly Ala Val Gly Asp Gly 5 10 15 Lys His Asp Cys Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala Ala Cys 20 25 30 Lys Asn Pro Ser Ala Met Leu Leu Val Pro Gly Ser Lys Lys Phe Val 35 40 45 Val Asn Asn Leu Phe Phe AsnGly Pro Cys Gln Pro His Phe Thr Phe 50 55 60 Lys Val Asp Gly IleIle Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys 65 70 75 80 Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu 85 90 95 Met Gly Lys Gly Val Ile Asp Gly Gln Gly Lys Gln Trp Trp Ala Gly 100 105 110 Gln Cys Lys Trp Val Asn Gly Arg GluIle CysAsn Asp Arg Asp Arg 115 120 125 Pro Thr Ala IleLys PheAsp Phe Ser Thr Gly Leu I le-Ile-Gln-Gly 130 135 140 Leu Lys Leu Met Asn Ser Pro Glu Phe-His-Leu-Val-Phe-Gly Asn Cys 145 150 155 160 Glu Gly Val Lys Ile Ile Gly Ile Ser Ile Thr Ala Pro Arg-Asp Ser 165 170175 Pro Asn Thr Asp Gly Ile Asp Ile Phe Ala Ser Lys Asn Phe His L u 180 185 190 Gln Lys Asn Thr Ile Gly Thr Gly Asp Asp Cys Val Ala Ile Gly Thr 195 200 205 Gly S r Ser Asn Il Val Ile Glu Asp Leu Ile Cys Gly Pro Gly His 210 215 220 Gly Ile Ser Ile Gly S r Leu Gly Arg Glu Asn Ser Arg Ala Glu Val 225 230 235 240 Ser Tyr Val His Val Asn Gly Ala Lys Phe Il Asp Thr Gln Asn Gly 245 250 255 Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser Gly Met Ala Ser His Ile 260 265 270 Ile Tyr Glu Asn Val Glu Met Il Asn Ser Glu Asn Pro Ile Leu Ile 275 280 285 Asn Gln Phe Tyr Cys Thr Ser Ala Ser Ala Cys Gln Asn

Gln Arg Ser 290 295 300 Ala Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser 305 310 315 320 Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser Asp Ser Met Pro Cys 325 330 335 Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser Gly Lys 340 345 350 Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gly His 355 360 365 Val Ile Pro Ala Cys Lys Asn Leu Ser Pro Ser Ala Lys Arg Lys Glu 370 375 380 Ser Lys Ser His Lys His Pro Lys Thr Val Met Val Glu Asn Met Arg 385 390 395 400 Ala Tyr Asp Lys Gly Asn Arg Thr Arg Ile Leu Leu Gly Ser Arg Pro 405 410 415 Pro Asn Cys Thr Asn Lys Cys His Gly Cys Ser Pro Cys Lys Ala Lys 420 425 430 Leu Val Ile Val His Arg Ile Met Pro Gln Glu Tyr Tyr Pro Gln Arg 435 440 445 Trp Ile Cys Ser Cys His Gly Lys Ile Tyr His Pro 450 455 460 array number: -- length [of three arrays]: -- mold [of 1542 arrays]: -- nucleic-acid-topology: -- kind [of straight chain-like array]: -- cDNAto mRNA Array : ATGGCCATGA AATTAATTGC TCCAATGGCC TTTCTGGCCA TGCAATTGAT TATAATGGCG 60 GCAGCAGAAG ATCAATCTGC CCAAATTATG TTGGACAGTG TTGTCGAAAA ATATCTTAGA 120 TCGAATCGGA GTTTAAGAAA AGTTGAGCAT TCTCGTCATG ATGCTATCAA CATCTTCAAT 180 GTGGAAAAAT ATGGCGCAGT AGGCGATGGA AAGCATGATT GCACTGAGGC ATTTTCAACA 240 GCATGGCAAG CTGCATGCAA AAACCCATCA GCAATGTTGC TTGTGCCAGG CAGCAAGAAA 300 TTTGTTGTAA ACAATTTGTT CTTCAATGGG CCATGTCAAC CTCACTTTAC TTTTAAGGTA 360 GATGGGATAA TAGCTGCGTA CCAAAATCCA GCGAGCTGGA AGAATAATAG AATATGGTTG 420 CAGTTTGCTA AACTTACAGG TTTTACTCTA ATGGGTAAAG GTGTAATTGA TGGGCAAGGA 480 AAACAATGGT GGGCTGGCCA ATGTAAATGG GTCAATGGAC GAGAAATTTG CAACGATCGT 540 GATAGACCAA CAGCCATTAA ATTCGATTTT TCCACGGGTC TGATAATCCA AGGACTGAAA 600 CTAATGAACA GTCCCGAATT TCATTTAGTT TTTGGGAATT GTGAGGGAGT AAAAATCATC 660 GGCATTAGTA TTACGGCACC GAGAGACAGT CCTAACACTG ATGGAATTGA TATCTTTGCA 720 TCTAAAAACT TTCACTTACA AAAGAACACG ATAGGAACAG GGGATGACTG CGTCGCTATA 780 GGCACAGGGT CTTCTAATAT TGTGATTGAG GATCTGATTT GCGGTCCAGG CCATGGAATA 840 AGTATAGGAA GTCTTGGGAG GGAAAACTCT AGAGCAGAGG TTTCATACGT GCACGTAAAT 900 GGGGCTAAAT TCATAGACAC ACAAAATGGA TTAAGAATCA AAACATGGCA GGGTGGTTCA 960 GGCATGGCAA GCCATATAAT TTATGAGAAT GTTGAAATGA TAAATTCGGA GAACCCCATA 1020 TTAATAAATC AATTCTACTG CACTTCGGCT TCTGCTTGCC AAAACCAGAG GTCTGCGGTT 1080 CAAATCCAAG ATGTGACATA CAAGAACATA CGTGGGACAT CAGCAACAGC AGCAGCAATT 1140 CAACTTAAGT GTAGTGACAG TATGCCCTGC AAAGATATAA AGCTAAGTGA TATATCTTTG 1200 AAGCTTACCT CAGGGAAAAT TGCTTCCTGC CTTAATGATA ATGCAAATGG ATATTTCAGT 1260 GGACACGTCA TCCCTGCATG CAAGAATTTA AGTCCAAGTG CTAAGCGAAA AGAATCTAAA 1320 TCCCATAAAC ACCCAAAAAC TGTAATGGTT GAAAATATGC GAGCATATGA CAAGGGTAAC 1380 AGAACACGCA TATTGTTGGG GTCGAGGCCT CCGAATTGTA CAAACAAATG TCATGGTTGC 1440 AGTCCATGTA AGGCCAAGTT AGTTATTGTT CATCGTATTA TGCCGCAGGA GTATTATCCT 1500 CAGAGGTGGA TATGCAGCTG TCATGGCAAA ATCTACCATC CA 1542 array number: -- length [of four arrays]: -- mold [of 1380 arrays]: -- nucleic-acid-topology: -- kind [of straight chain-like array]: -- cDNA to mRNA array: -- GCTATCAACA TCTTCAATGT GGAAAAATAT GGCGCAGTAG GCGATGGAAA GCATGATTGC 60 ACTGAGGCAT TTTCAACAGC ATGGCAAGCT GCATGCAAAA ACCCATCAGC AATGTTGCTT 120 GTGCCAGGCA GCAAGAAATT TGTTGTAAAC AATTTGTTCT TCAATGGGCC ATGTCAACCT 180 CACTTTACTT TTAAGGTAGA TGGGATAATA GCTGCGTACC AAAATCCAGC GAGCTGGAAG 240 AATAATAGAA TATGGTTGCA GTTTGCTAAA CTTACAGGTT TTACTCTAAT GGGTAAAGGT 300 GTAATTGATG GGCAAGGAAA ACAATGGTGG GCTGGCCAAT GTAAATGGGT CAATGGACGA 360 GAAATTTGCA ACGATCGTGA TAGACCAACA GCCATTAAAT TCGATTTTTC CACGGGTCTG 420ATAATCC AAG-GACTGAAACT-AATGAACAGT CCCGAATTTC-ATTTAGTTTT TGGGAATTGT

480GAGGGAGTAA AAATCATCGG-CATTAGTATT ACGGCACCGA-GAGACAGTCC
TAACACTGAT 540GGAATTGATA TCTTTGCATC-TAAAAACTTT
CACTTACAAA-AGAACACGAT AGGAACAGGG 600GATGACTGCG
TCGCTATAGG-CACAGGGTCT TCTAATATTG-TGATTGAGGA TCTGATTTGC
660GGTCCAGGCC ATGGAATAAG TATAGGAAGT CTTGGGAGGG AAAACTCTAG
AGCAGAGGTT 720TCATACGTGC ACGTAAATGG GGCTAAATTC ATAGACACAC
AAAATGGATT AAGAATCAAA 780ACATGGCAGG GTGGTTCAGG CATGGCAAGC
CATATAATTT ATGAGAATGT TGAAATGATA 840AATTCGGAGA ACCCCATATT
AATAAATCAA TTCTACTGCA CTTCGGCTTC TGCTTGCCAA 900AACCAGAGGT
CTGCGGTTCA AATCCAAGAT GTGACATACA AGAACATACG TGGGACATCA
960GCAACAGCAG CAGCAATTCA ACTTAAGTGT AGTGACAGTA
TGCCCTGCAAAGATATAAAG 1020 CTAAGTGATA TATCTTTGAA GCTTACCTCA
GGGAAAATTG CTTCCTGCCT TAATGATAAT 1080 GCAAATGGAT ATTTCAGTGG
ACACGTCATC CCTGCATGCA AGAATTTAAG TCCAAGTGCT 1140 AAGCGAAAAG
AATCTAAATC CCATAAACAC CCAAAAACTG TAATGGTTGA AAATATGCGA 1200
GCATATGACA AGGGTAACAG AACACGCATA TTGTTGGGGT CGAGGCCTCC GAATTGTACA
1260 AACAAATGTC ATGGTTGCAG TCCATGTAAG GCCAAGTTAG TTATTGTTCA
TCGTATTATG 1320 CCGCAGGAGT ATTATCCTCA GAGGTGGATA TGCAGCTGTC
ATGGCAAAAT CTACCATCCA 1380 array number: -- length [of five arrays]: -- mold [of
1733 arrays]: -- nucleic-acid-topology: -- kind [of straight chain-like array]: -- cDNA to
mRNA array: -- AGTTGAGTTC GAGACAAGTA TAGAAAGAAT TTTCTTTTAT TAAAATGGCC
ATGAAATTAA 60 TTGCTCCAAT GGCCTTTCTG GCCATGCAAT TGATTATAAT
GGCGGCAGCA GAAGATCAAT 120 CTGCCCAAAT TATGTTGGAC AGTGTTGTCG
AAAAATATCT TAGATCGAAT CGGAGTTTAA 180 GAAAAGTTGA GCATTCTCGT
CATGATGCTA TCAACATCTT CAATGTGGAA AAATATGGCG 240 CAGTAGGCGA
TGGAAAGCAT GATTGCACTG AGGCATTTTC AACAGCATGG CAAGCTGCAT 300
GCAAAAACCC ATCAGCAATG TTGCTTGTGC CAGGCAGCAA GAAATTTGTT GTAAACAATT
360 TGTTCTTCAA TGGGCCATGT CAACCTCACT TTACTTTTAA GGTAGATGGG
ATAATAGCTG 420 CGTACCAAAA TCCAGCGAGC TGGAAGAATA ATAGAATATG
GTTGCAGTTT GCTAAACTTA 480 CAGGTTTTAC TCTAATGGGT AAAGGTGTAA
TTGATGGGCA AGGAAAACAA TGGTGGGCTG 540 GCCAATGTAA ATGGGTCAAT
GGACGAGAAA TTTGCAACGA TCGTGATAGA CCAACAGCCA 600 TTAAATTCGA
TTTTTCCACG GGTCTGATAA TCCAAGGACT GAAACTAATG AACAGTCCCG 660
AATTTCATTT AGTTTTTGGG AATTGTGAGG GAGTAAAAAT CATCGGCATT AGTATTACGG
720 CACCGAGAGA CAGTCCTAAC ACTGATGGAA TTGATATCTT TGCATCTAAA
AACTTTCACT 780 TACAAAAGAA CACGATAGGA ACAGGGGATG ACTGCGTCGC
TATAGGCACA GGGTCTTCTA 840 ATATTGTGAT TGAGGATCTG ATTTGCGGTC
CAGGCCATGG AATAAGTATA GGAAGTCTTG 900 GGAGGGAAAA CTCTAGAGCA
GAGGTTTCAT ACGTGCACGT AAATGGGGCT AAATTCATAG 960 ACACACAAAA
TGGATTAAGA ATCAAAACAT GGCAGGGTGG TTCAGGCATG GCAAGCCATA 1020
TAATTTATGA GAATGTTGA A-ATGATAAATT-CGGAGAACCC CATATTAATA-AATCAATTCT
1080ACTGCACTTC GGCTTCTGCT TGCCAAAACC-AGAGGTCTGC
GGTTCAAATC-CAAGATGTGA 1140CATACAAGAA CATACGTGGG
ACATCAGCAA-CAGCAGCAGC AATTCAACTT-AAGTGTAGTG 1200ACAGTATGCC
CTGCAAAGAT ATAAAGCTAA-GTGATATATC TTTGAAGCTT-ACCTCAGGGA 1260
AAATTGCTTC CTGCCTTAAT GATAATGCAA ATGGATATTT CAGTGGACAC GTCATCCCTG
1320 CATGCAAGAA TTTAAGTCCA AGTGCTAAGC GAAAAGAATC TAAATCCCAT
AAACACCCAA 1380 AAACTGTAAT GGTTGAAAAT ATGCGAGCAT ATGACAAGGG
TAACAGAACA CGCATATTGT 1440 TGGGGTCGAG GCCTCCGAAT TGTACAAACA
AATGTCATGG TTGCAGTCCA TGTAAGGCCA 1500 AGTTAGTTAT TGTTCATCGT
ATTATGCCGC AGGAGTATTA TCCTCAGAGG TGGATATGCA 1560 GCTGTCATGG

CAAAATCTAC CATCCATAAT GAGATACATT GAAACTGTAT GTGCTAGTGA 1620
ATATTCTTGT GGTACAATAT TAGAACTGAT ATTGAAAATA AATCATCAAT GTTTCTAAGG
1680 CATTTATAAT AGATTATATT AATGGTTCAA AAAAAAAAAA AAAAAAAAAA AAA 1733

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] N of Japan cedar pollen-allergen Cry j II Amino acid sequence of an end to ten residues (A) . N of Japan cedar pollen-allergen Cry j II DNA expected from the amino acid s quence of an end to ten residues Array (B) . DNA of the probe for screening cDNA which carries out the code of Japan cedar pollen-allergen Cry j II Array (C) .
[Drawing 2] T7 Cry j II It is antigenicity 2 The result identified by the Western blot technique is shown using the blood serum of the Japan cedar pollinosis patient of a name. For BL21 (DE3) holding pMGEMEX-1 (negative control), and a lane 2, BL21 (DE3) which discovered T7 Cry j I, and a lane 3 are [a lane 1] T7Cry j II. A lane 4 is discovered BL21 (DE3) and Cry j I refined from Japan cedar pollen. It is shown, respectively. A B It is only that the patients in whom a blood serum originates differ, and others are the same.
[Drawing 3] The overlap peptide which covers all the amino acid sequences of Cry j II is shown.
[Drawing 4] The overlap peptide which covers all the amino acid sequences of Cry j II is shown.
[Drawing 5] The overlap peptide which covers all the amino acid sequences of Cry j II is shown.
[Drawing 6] The overlap peptide which covers all the amino acid sequences of Cry j II is shown.
[Drawing 7] At least one T of Cry j II The peptide containing a cell epitope is shown.
[Drawing 8] At least one T of Cry j II The peptide containing a cell epitope is shown.
[Drawing 9] T specific to the Cry j II allergen established from 18 Japan cedar pollinosis patients The significance index of the overlap peptide which a cell line recognizes, respectively is shown.